STUDIES ON THIN-LAYER CHROMATOGRAPHIC ANALYSIS OF INORGANIC IONS

SUMMARY

THESIS

SUBMITTED FOR THE DEGREE OF

Doctor of Philosophy

IN

APPLIED CHEMISTRY

BY

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LIST OF PUBLICATIONS

1. A. Mohammad , S.Tiwari and **J.P.S.Chahar**. *Thin-Layer Chromato graphic Separation , Identification and Determination of Certain Anions* , ***J.Chromatogr. Sci.*** **33 (1995) 143 .**
2. A. Mohammad . S. Tiwari , **J.P.S. Chahar** and S. Kumar , *Water-in-Oil Microemulsion as Mobile Phase in Thin-Layer Chromatographic Retention Studies of Anions* , ***J.Am. Oil Chem. Soc.*** **72 (12) (1995) 1533 .**
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5. A. Mohammad and **J.P.S.Chahar**, *Colorimetric Determination of Ag (I) in Ores With Preliminary TLC Separation from Associated Metal Ions,* ***J.Assoc. Off. Anal. Chem.*** (Communicated) .
6. A. Mohammad and **J.P.S.Chahar**, *Thin-Layer Chromatography in the Analysis of d-block Elements and Their Anions* , ***J.Ind. Chem. Soc.*** (Communicated) .

SUMMARY

The study was aimed to develop thin layer chromatographic systems for the identification, separation and determination (semi-quantitative / quantitative) of inorganic anions (halides, halates, CN - containing and oxyanions of metals) and cations of p - and d - block metals. Ions were selected on the basis of their industrial, environmental, geological, biological and agricultural importance. To achieve the target, the work presented in this thesis was performed. Several thin layer chromatographic systems were developed by using; (i) plain commonly available adsorbents i.e silica gel, cellulose microcrystalline, aluminium oxide, kieselguhr, kaolin; (ii) binary mixtures of these adsorbents in various ratios and (iii) impregnated layers with complexing salts.The layer thickness of 0.25 mm has been kept constantly. TLC plates were prepared manually by using a TLC applicator (Toshniwal, India). Mobile phase plays a crucial role to make the chromatographic systems more selective. Low cost solvents (double distilled water, acetone, mineral and carboxylic acids, ammonia and its salts; and their binary mixtures) were used as mobile phases. Mode of chromatogram development strongly affect the development time. Ascending, the most widely used mode has been adopted for the entire thin layer chromatographic studies. Some important separations were achieved by employing newly developed chromatographic systems followed by the quantitative studies. The results have been summarized in seven chapters, discussed briefly as :

CHAPTER - I GENERAL INTRODUCTION

This chapter provides a wider information about the general aspects of chemistry, analytical chemistry, chromatography and thin - layer chromatography (TLC). Chromatography was classified on the basis of phase distribution process. Some chromatographic techniques have been enlisted alongwith their origin and salient features (Table 1.1). Analyte species have also been classified (Figure 1.1).

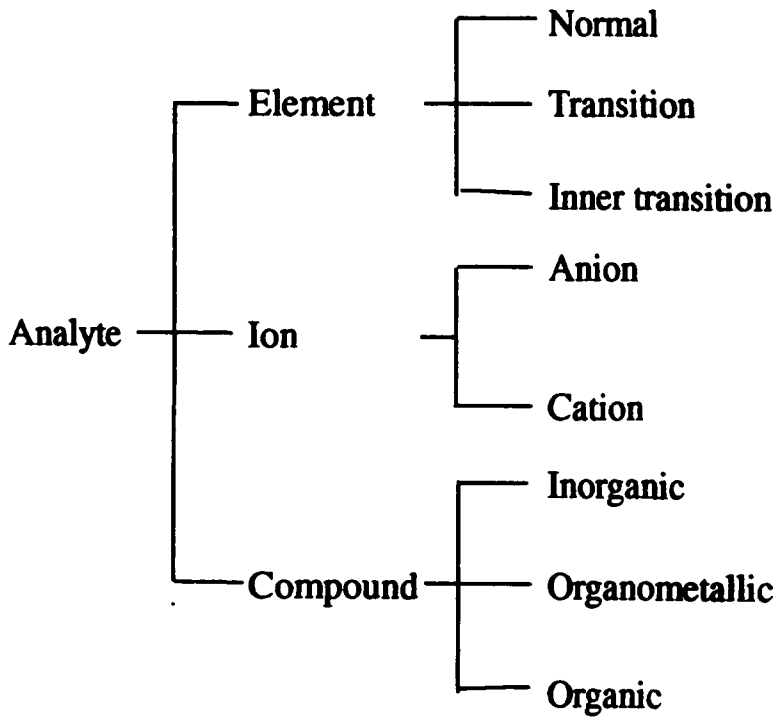

Figure 1.1: Classification of Analyte Species

Yearwise developments in the history of TLC have been summarized (Table 1.2). Semi - empirical models of chromatography correlating the R_F (retardation factor) with various physical parameters have been described briefly under the heading , 'Theory and Principle of TLC'. All the basic steps of a TLC analysis ranging from sample preparation to quantitative determination, have been discussed in this chapter.

CHAPTER - II : THIN - LAYER CHROMATOGRAPHY OF INORGANIC IONS SINCE 1980

This chapter presents a literature survey for last seventeen years (1980 - 1996) on the thin layer chromatographic analysis of inorganic ions. There are two hundred and fifty-four references cited in this chapter. Relative studies have been made to compare the work done (number of publications) on the TLC of inorganic anions and cations (Figure 2.1). Figure 2.2 shows that most of publications dealing with the TLC of inorganic ions, have been appeared in Journal of planar chromatography.

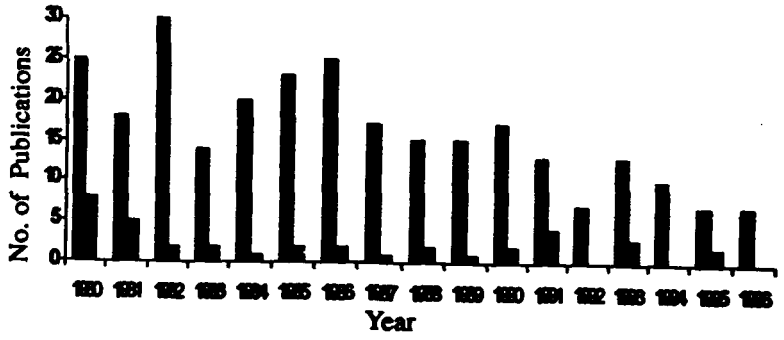

Figure 2.1 : Relative Study of Work Done on the TLC of Inorganic of Anions and Cations

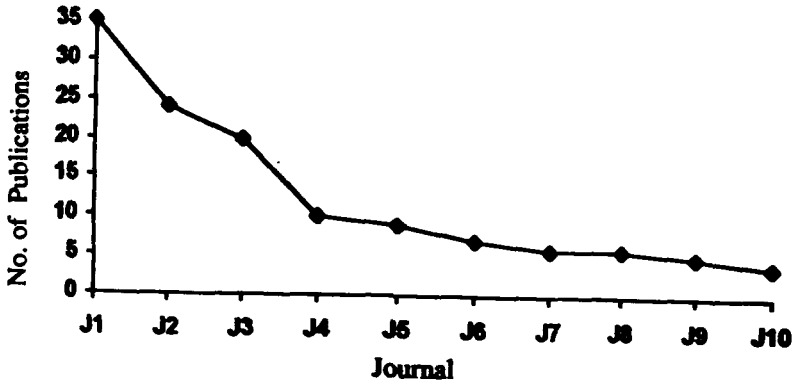

Figure 2.2 : Top Ten Journals Regarding the Number of Publications on TLC of Inorganic Ions (1980-1996)

CHAPTER III: TLC SEPARATION, INDENTIFICATION AND DETERMINATION OF CERTAIN ANIONS

This chapter describes the retention studies of ninteen inorganic anions on plain, impregnated and mixed sorbent phases of silica gel, cellulose microcrystalline kaolin, alumina and kieselguhr G have been examined in aqueous solvent systems. Analytical potential of water, a non-toxic eluant has been explored for its applicability as a simple, inexpensive and easily available developer in liquid layer chromatography. Better separation possibilities of anions on cellulose with HCl-acetone (1:9) eluant and on cellulose + alumina (1:2) mixed bed with water, were realized.Some ternary separations are shown in figure 3.1.

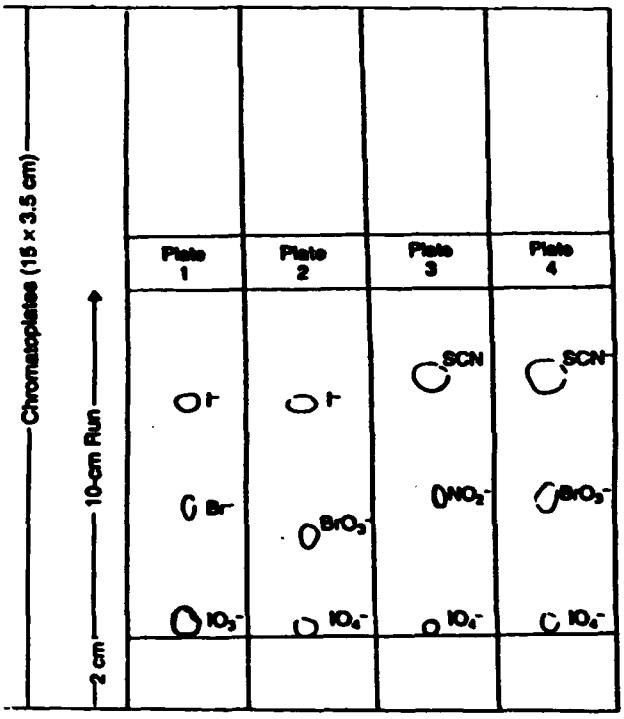

Figure 3.1 :Diagram of some ternary separations: 1, IO_3^- -BR^--I^-; 2, IO_4^- -BRO_3^- -I^-; 3, IO_4^- -NO_2^- -SCN^-; 4, IO_4^- -BrO_3^- -SCN^-. Conditions: stationary phase, cellulose; mobile phase, HCl-acetone (1:9).

The limits of identification for I^-, Br^-,NO_2^-, SCN^-, BrO_3^-,IO_3^-, IO_4^-, CrO_4^{2-} and PO_4^{3-} have also been determined semi quantitative determination I^-,Br^- and NO_2^- have been attempted by spot-area measurement method.

CHAPTER IV : W/O MICROEMULSION AS MOBILE PHASE IN THIN LAYER CHROMATOGRAPHIC RETENTION STUDIES OF ANIONS

A water-in-oil microemulsion, consisting of SDS/1-petanol/water/n-heptane, has been used as mobile phase for the separation of inorganic anions from their binary mixtures. The weight ratio, SDS/1- pentanol was kept constant at 1/2.46 for all compositions. The retentions efficiency of anions on layers of silica gel G, alumina , cellulose microcrystalline, kieselguhr G and mixtures of kieselguhr and cellulose in 4:1 and 3:2 ratios, has been examined with the W/O microemulsion system. Thin layers of kieselguhr were most useful for differential migration of anions. Quantitative separation of IO_4^- from accompanying ions, limits of determination and dilution of few anions have been reported. The effects of amines, phenols and heavy matels on the separation efficacy of IO_4^- also have been investigated. Semi-quantitation of IO_4^- by peak-height measurement has been performed (Figure 4.1).

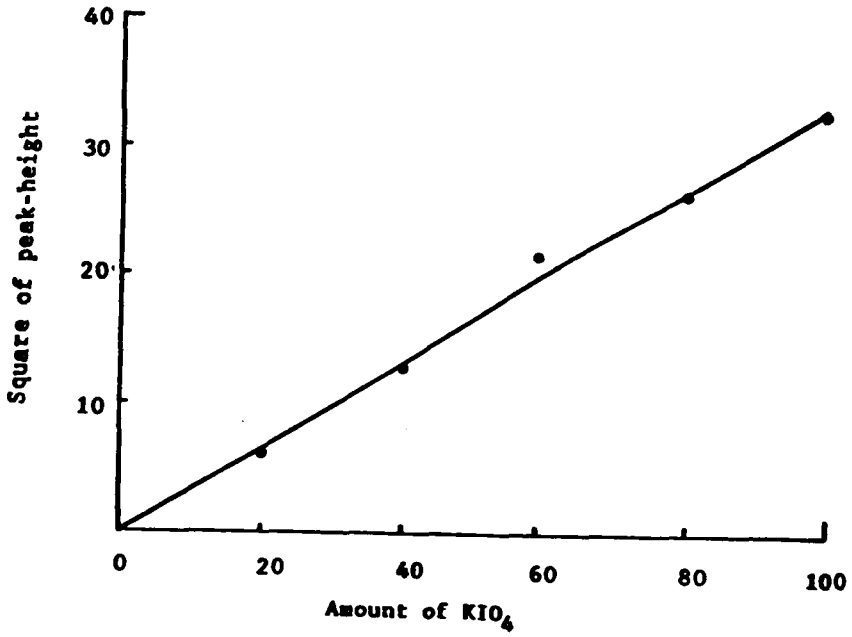

Figure 4.1: Standard calibration curve for semiquantitative determination of IO_4^- by peak-height measurement method.

CHAPTER - V : TLC SEPARATION, COLORIMETRIC DETERMINATION AND RECOVERY OF THIOCYANATE FROM PHOTOGENIC WASTE, RIVER AND SEA WATERS

This chapter summarizes the results of thin layer chromatigraphic retention studies of some inorganic anions (SCN^-, NO_2^-, MnO_4^-, CrO_4^{2-}, $Cr_2O_7^{2-}$, MoO_4^{2-}, $Mo_7O_{24}^{6-}$, $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$) and metal ions (Fe^{3+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, Hg^{2+}, UO_2^{2+}, VO^{2+}, Ag^+, Mn^{2+} and Bi^{3+}).Inorganic ions were chromatographed on plain and mixed adsorbents containing cellulose and/or kieselguhr using mixures of 0.1 M NH_4OH and CH_3COCH_3 in various ratios as mobile phases. The effect of proportional composition of mobile phase on the hR_F values of inorganic anions was examined. Cations remained at the point of application. Some anion - cation separations (Ag^+ - SCN^-, Fe^{3+} - SCN^-, Co^{2+} - SCN^-) were achieved. Effect of additives and pH was examined on the TLC detection of SCN^-.

The proposed TLC system was coupled to the spectrophotometry of SCN^- using Fe^{3+} - SCN^- color reaction in acidic medium. The optical density was measured at 460 nm and the Beer's law was followed upto 11.18 ppm concentration of SCN^-.

To test the applicability, proposed TLC - colorimetric method was applied to the fortified samples of photogenic waste, river water and seawater. During entire experimental work, relative standard deviation varies from 2.124 to 10.442 pph.

CHAPTER - VI : A NEW CHROMATO - IODOMETRIC METHOD FOR THE SEPARATION AND DETERMINATION OF IODIDE AND ITS OXYANIONS

This chapter encapsulates the results showing the retention behavior of I^-, IO_3^- and IO_4^- ions on plain sorbent phases (silica gel G, cellulose microcrystalline, alumina G and kieselguhr G) using mixtures of 0.1 M NH_4OH and CH_3COCH_3 as mobile phases. The chromatographic system consisting of alumina and 0.1 M NH_4OH-CH_3COCH_3 (9:1) was found to be best as it provides more compact and well resolved spots of anions. Effect of time was examined on the detection of IO_4^- in a ternary mixture with IO_3^- and I^- (Figure 6.1).

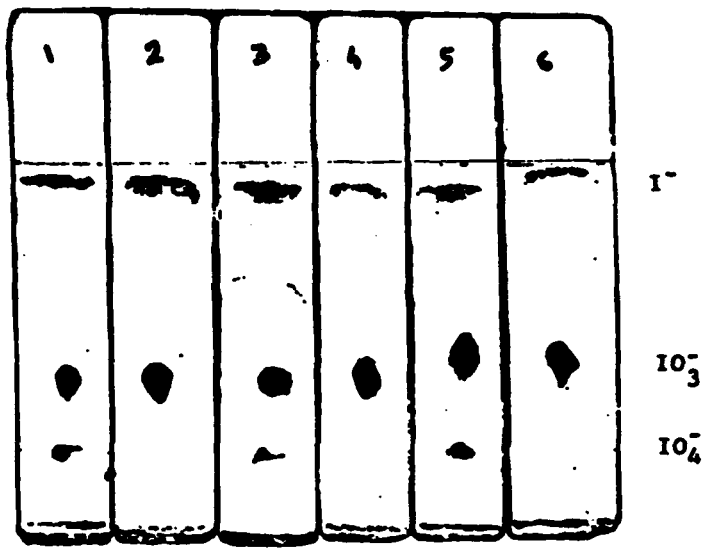

Figure 6.1: TLC Detection of I^-, IO_3^- and IO_4^- under optimized pH and Mixing Time. pH; 4.0 (Plate No. 1,2), 7.0(3,4) and 10.0(5,6); Mixing Time; 30 Min(1), 35 Min(2), 60 Min(3), 65 Min(4), 75 Min(5) and 80 Min(6).

A new iodometric method was developed for the quantification of I^-, IO_3^- and IO_4^-. The titrant was 2.0% aqueous acetone for all analytes. The proposed TLC - iodometric method was applied to recover iodide, iodate and periodate from fortified water samples. The LOQ for I^-, IO_3^- and IO_4^- were 76.4, 16.3 and 16.6 μg respectively.

CHAPTER - VI I : COLORIMETRIC DETERMINATION OF Ag(I) IN ORES WITH PRELIMINARY TLC SEPARATION FROM ASSOCIATED METAL IONS

This chapter encapsulates the results of TLC separation of metal ions on layers of cellulose microcrystalline, alumina G and their binary mixtures. Aqueous solutions of NH_3, AcOH, AcONa, $AcONH_4$ and $NaNO_3$ were used as mobile phase. Ag^+ was found separated from other metal ions on mixed layer using 1.0 M NH_4OH as mobile phase. Some important binary, ternary and quaternary separations were achieved. Effect of acid - base equilibrium was examined on the retention behavior of Ag^+. Figure 7.2 shows the formation of double spot of Ag^+.

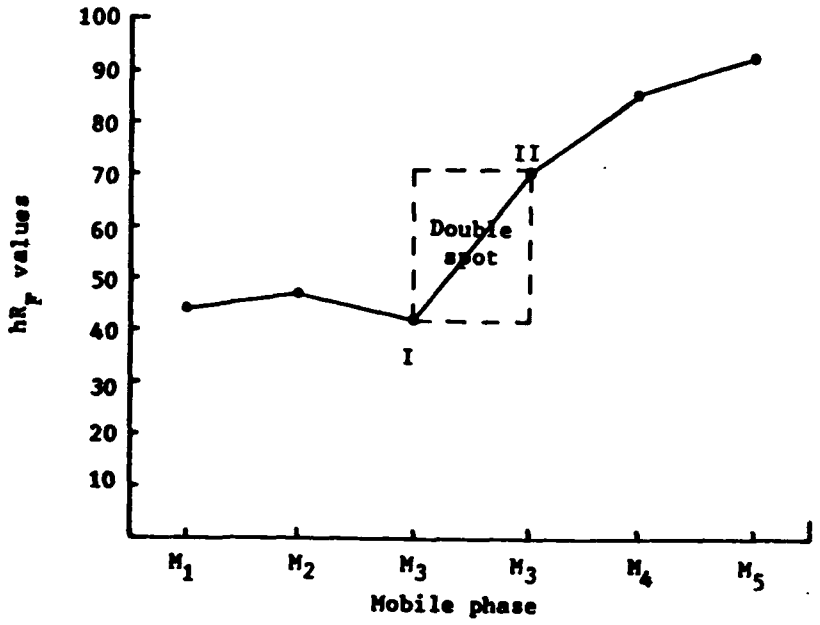

Figure 7.2: Formation of double spot of Ag^+. M_1-M_5 are mixed mobile phases containing AcOH and NH_4OH in 10:0, 7:3, 1:1, 3:7 and 0:10 respectively.

After TLC separation, Ag^+ was quantitatively determined by using dithzone solution (in acetone) as chromogenic reagent. The optical density was measured at 480 nm to sketch the standard calibration curve (Figure 7.3). The TLC - colorimetric method was applied to the horn silver (AgCl) and argentite (Ag_2S) for the recovery of Ag^+.

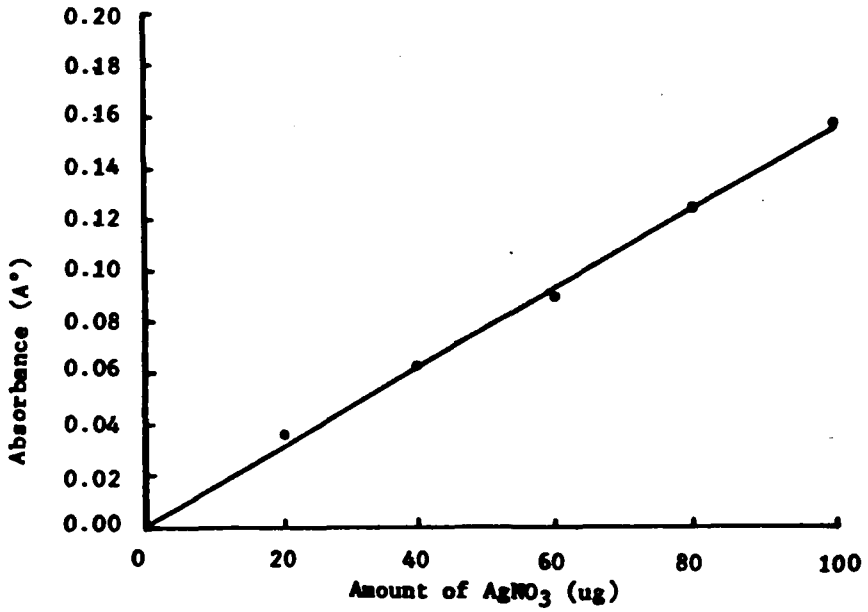

Figure 7.3: Standard calibration curve for the colorimetry of Ag(I).

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1997

DEDICATED

to (Late)**Professor M. Qureshi,**

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CERTIFICATE

Certified that the work incorporated in this thesis is the original contribution of ***Mr. Jay Pal Singh Chahar*** and is suitable for submission to the award of Ph. D degree.

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CONTENTS

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This is an important moment of my life when I have to acknowledge all those concerned with this work. I do not have sufficient words to frame a sentence in the honor of **Dr. Ali Mohammad**, my supervisor. **Dr. Ali Mohammad** (Reader, Department of Applied Chemistry, A.M.U., Aligarh) guided me, supported me and encouraged me constantly during my research period. I am grateful to **Prof.Mohammad Ajmal**, Chairman of the Department, for providing research facilities.

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(JAY PAL S. CHAHAR)

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CHAPTER -I

GENERAL INTRODUCTION

Chemistry, the branch of science is concerned with the studies of properties, composition and structure of matter. The term 'Chemistry' was originated from the word 'Chemia' which means black color. The soil of Egypt was called as chemia since it was black in color. Chemistry deals with a wide field of knowledge, so for the ease of studies it became imperative to classify it into various branches e.g. inorganic , organic , physical , industrial, agricultural and analytical chemistry.

1.1 ANALYTICAL CHEMISTRY

Analytical chemistry has emerged among the branches of science to be the most interdisciplinary. It is concerned with the theory and practice of methods used to determine the composition of matter. Analytical chemistry is also concerned with the techniques employed in chemical analysis. The analysis may either be qualitative (study of physical / chemical nature of analyte species) or quantitative (determination of how much of a particular substance is present in a sample) . Analyte species may be classified as shown in Figure 1.1 .

In recent years ,technological developments has create analytical problems which demand increasingly sophisticated instrumentation and wider knowledge for their solution. As a result of advancements made, numerous instrumental and non-instrumental techniques (e.g. gravimetry, titrimetry, solvent extraction, spectroscopy , polarography, potentiometry, electrophoresis and chromatography) are available today for analysts' use.

1.2CHROMATOGRAPHY

The term 'Chromatography' is derived from two Greek words '*Chroma*' and '*Graphy*' meaning *color* and *writing* respectively. Hence, the chromato-

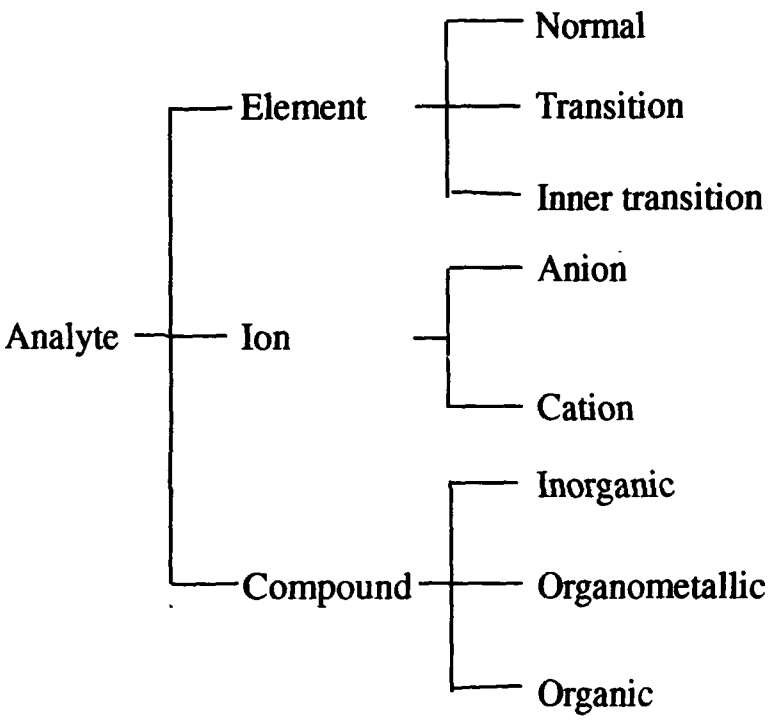


Figure 1.1: Classification of Analyte Species

graphy is a branch of analytical chemistry where the studies are based on color. Although, meaning of the term is largely understood by chemists, a good definition of chromatography is difficult to formulate. It is a collective term to methods which appear diverse in some regards but share certain common features. A definition should emphasize that components of sample are distributed between two phases but this alone is inadequate because we do not wish the term to embrace all the separation processes. Chromatography defined by Cassidy (1) and Keulemans (2) received wide acceptance in the past . However, the IUPAC has recently defined chromatography as ; *A method used primarily for the separation of components of a sample, in which the components are distributed between two phases, one of which is stationary , while the other moves. The stationary phase may be a solid, a solid supported liquid or a gel. The mobile phase may be a liquid or a gas.* The separation occurs because each analyte distributes differently between both the phases.

1.3 HISTORY OF CHROMATOGRAPHY

At about the end of 19th century , Davy observed the compositional changes in crude petroleum when it came in contact with rocks. A Russian botanist M. Tswett was attracted towards the fact that the crude petroleum is derived from the plants which were burried millions of years ago under the earth's crust while a rock is not much different from $CaCO_3$ from the chemical point of view . Being inspired from Davy's observation , M. Tswett packed a narrow glass tube with $CaCO_3$ and passed petroleum ether extract of chlorophyll and other plant pigments through that column . M.Tswett

Table 1.1: History and Distinguish Characteristics of some Chromato graphic Techniques .

Originator	Technique	Salient features
M.S.Tswett (1906)	Adsorption Chromatography	Solid S.P.[1] (column) ; flow of liquid mobile phase under CA[2]; adsorption phenomena.
P.Konig (1937)	Electrophoresis	Paper S.P.; liquid mobile phase moves under FF (electrostatic force) .
N.A.Izmailov and M.S.Schraiber (1938)	Thin-Layer Chromatography (Adsorption)	Solid S.P. (planar) ; liquid mobile phase flows under CA; adsorption .
A.J.P. Martin and R.L.M.Synge (1941)	Partition Chromatography	Solid supported liquid S.P.; liquid mobile phase flows under CA; partition phenomena.
A.J.P.Martin, R.Consden and A.H.Gordon (1944)	Paper Chromatography	Paper supported liquid S.P.; liquid mobile phase flows under CA; liquid-liquid partition .
L.C.Craig (1944)	Countercurrent Chromatography	Liquid-liquid partition Chromatography .
Barrer (1945)	Gel-Permeation Chromatography	Solid S.P.(column); Solvent moves under CA; size-exclusion phenomena .
S.Claesson (1946)	Gas-Solid Chromatography	Solid (column) S.P.; gas mobile phase moves under FF[3] .
Mayer and Thompkins	Ion-Exchange Chromatography	Solid (ion-exchange resin) S. P. in the shape of column; liq-

Originator	Technique	Salient Features
(1947)		uid mobile phase moves under CA; ion-exchange phenomena
J.G.Kirchner; J.M.Miller and G.J.Keller (1951)	Thin-Layer Chromatography (partition)	Solid Supported liquid (planar) S.P. ; liquid mobile phase flows under CA; partition phenomena .
A.T. James and A.J.P.Martin (1952)	Gas-liquid Chromatography	Bonded liquid (column) S. P. ; gas mobile phase flows under FF; partition phenomena.
A.T.James and A.J.P.Martin (1952)	High-performance liquid Chromatography	Bonded liquid(column)S. P.; liquid mobile phase moves under FF; modified partition phenomena .
H. Small ,T.S. Stevens and W.C.Bauman (1975)	Ion Chromatography	Bonded liquid (column) S. P. ; liquid mobile phase moves under FF ; suitable for ions .
A.Zlatkis and R.E.Kaises (1976)	High - performance Thin-layer Chromatography	Bonded liquid (planar) S. P.; liquid mobile phase moves under CA; modified partition phenomena; instrumental technique with computerized documentation .
E.Tyihak, E.Mincsovics and H.Kalasz (1979)	Over-pressurized layer Chromatography	Solid (planar) S. P. ; liquid mobile phase moves under FF. OPLC is TLC with a constant optimized mobile phase flow speed .

found plant pigments separated and he published two research papers (3,4). Tswett's work was realized in 1931 by a prominent organic chemist R.Kuhn and his co-workers when they used chromatographic system similar to that used by Tswett and separated natural products (5,6). This research attracted more attention and the adsorption phenomena was started to be used in the field of natural product chemistry . When chromatography became capable of solving critical analytical problems , analysts were attracted towards it and made improvements in this field with regards to the separation efficiency , selectivity and sensitivity of chromatographic system , lesser retention time etc. As a result , Various chromatographic techniques have been developed and of them , some are presented in Table 1.1 .

1.4 CLASSIFICATION OF CHROMATOGRAPHY

All the known types of chromatography can be put into four groups ; (a) liquid-solid , (b) gas-solid , (c) liquid-liquid and (d) gas-liquid . Considering various classification factors e.g. nature and physical state of stationary and mobile phases , mode of mobile phase movement and shape of stationary phase . chromatographic techniques may also be classified . Besides these factors , chromatography may also be classified on the basis of phase distribution process , as described below ;

***(a) Adsorption Chromatography*:** Adsorption is the tendency of molecules , ions or atoms in solution to interact with the surface of a solid. The chromatography involving adsorption of analyte species as retention phenomenon on a stationary phase of a surface active granular solid is known as adsorption chromatography . The surface of an adsorbent has rigid structure and free valencies to make the chromatographic system more useful

to separate geometrical and structural isomers . Silica has been the most common adsorbent since long . At present , a variety of silicas and other customary materials such as $CaCO_3$, $MgCO_3$,kieselguhr , alumina , starch, cellulose and organic polymers are available to use as stationary phase .

The use of more polar stationary phases with less polar mobile phases was considered to be the normal phase adsorption chromatography . A different approach utilizing non-polar adsorbents and more polar mobile phases has become dominant in recent years and is termed as reversed phase chromatography .

***(b) Partition Chromatography*:** The chromatography in which the distribution of solute takes place between two nearly immiscible liquids (stationary and mobile phases) is known as partition chromatography . The stationary liquid is supported by a highly porous solid of large surface area. In 1941 , Martin and Synge (7,8) employed silica gel as a solid support to immobilize water as stationary phase in a chromatographic column. Chemically bonded stationary phases are viewed by many chromatographers as liquid films on solid supports , or at least analogs there of ; reversed phase operations with such column packings are then treated as liquid-liquid partition. The bonded phases eliminate problems related to stripping of stationary liquid from the column by the mobile phase .In principle , this is circumvented by pre-saturating the mobile phase with the stationary liquid , but difficulty ensues if it is desired to alter the composition of mobile phase during the experiment (i.e. gradient elution) .

(c) Ion-Exchange Chromatography : In this type of liquid chromatography , ion-exchange resins are used as stationary phase . A resin is a solid, insoluble co-polymer , capable of exchanging ions of similar charge present in a solution . The resins are prepared by introducing ionizable groups into an organic polymer matrix , of which the most common is the cross-linked polystyrene . A table of ion-exchange resins and their properties has been presented by Dorfner (9) . The major types of ion-exchange layers in use includes , polyester sheets coated with a mixture of silica gel and anion /cation exchange resin with an inert binder (Polygram ionex-5), polyethyleneimine (PEI) cellulose , diethylaminoethyl (DEAE) cellulose , mixed layer of DEAE cellulose and unmodified cellulose etc. .

(d) Size-Exclusion Chromatography : In size-exclusion chromatography , the stationary phase consists of small particles having pores. If certain molecules are sufficiently small to move into the pores ,they will be held up the stationary phase and those particles which are too large to enter the pores will pass the stationary phase readily on percolating the mobile phase. Size-exclusion chromatography is also known as gel-filtration chromatography or gel-permeation chromatography . This is of greater use in liquid chromatography for the separation of molecules having vapour pressure too small to be separated by the gas chromatography .

The process of size -exclusion is concerned with the distribution of solute between the aqueous phase within the gel and external water . Two important types of column materials useable in aqueous media are cross-linked dextrans (Sephadex G) and polyacrylamides (Bio-gel F) .

1.5 THIN-LAYER CHROMATOGRAPHY

TLC is a type of liquid chromatography in which the stationary phase is in the form of layer supported by glass plate, aluminium foil or plastic sheet. As originally developed in 1938 and still widely practiced today, classical capillary action TLC is an inexpensive, rapid, simple and highly effective analytical technique requiring little instrumentation. A suitable development chamber containing mobile phase and a TLC plate is all that required to carryout qualitative as well as quantitative analysis .TLC is highly selective and flexible because of the availability of a great variety of layer materials and wider choice of mobile phases. Following are some purposes for which the thin-layer chromatography is of help .

(i) Identification of substance.

(ii) Separation of two or more components of a mixture.

(iii) Determination of amount of a particular species present in a sample.

(iv) Preconcentration and preparation of a sample.

(v) Study of relative polarity of any solid-liquid or liquid-liquid phase.

1.6 HISTORY OF TLC

In 1966, Pelick et al. (10) presented a tabulation of significant developments in TLC and provided translation of classical studies made by Izmailov and Schraiber and by Stahl. In continuation, the history of TLC was reviewed by Stahl (11), Heftmann (12), Kirchner (13-15), Jork and Wimmer(16), Wintermeyer (17) and Sherma (18). From the historical point of view, countable achievements made in the history of TLC , are enlisted in Table 1.2 .

Table 1.2: Important Years in the History of TLC.

Year	Chromatographer(s) and their work	Ref.(s)
1938	Izmailov and Schraiber used unbound alumina layers and applied the drops of solvent to the plate to separate certain medicinal compounds. The procedure was called "drop chromatography" .	19
1949	Meinhard and Hall, using drop chromatography, separated Fe^{2+} and Zn^{2+} on microscope slides coated with alumina (adsorbent) + starch (binder) .	20
1951	Kirchner et al. used glass plates to support the layer, developed the plates by ascending technique, and coined the term chomatostrip for his layers .	21
1958	E. Stahl introduced the term chromatography and standardized the materials , procedures and nomenclature involved in TLC .	22,23
1965	Przybylowicz et al. discussed the importance of precoated TLC plates .	24
1976	Zlatkis and Kaiser modernized TLC in the form of a highly instrumental technique and named as high performance thin-layer chromatography (HPTLC).	25
1979	Tyihak et al. applied extra force for the movement of solvent and introduced over-pressurized layer chromatography (OPLC) .	26

1.7 THEORY AND PRINCIPLE OF TLC

In the liquid chromatography, the solvent phase moves through interparticular space available among the particles of stationary phase . When passes through the point (where the analyte has been spotted), the mobile phase tries to keep the solute with it. The migration of solute over stationary phase depends upon the relative interactions of stationary / mobile phase on the solute .In a TLC system , the R_F coefficient is a basic quantity used to express the migration of analyte species on the chromatoplate . It can be calculated as,

$$R_F = \frac{\text{Distance travelled by the analyte}}{\text{Distance travelled by the solvent front}}$$

Both the distances are measured from the point of application of analyte. In other words,

$$R_F = \frac{R_L + R_T}{2}$$

Where , R_L and R_T values are R_F of leading and trailing fronts of the spot respectively . The R_F values varies from 0.00 (analyte remain at the spotting line) to 0.99 (analyte migrates with the solvent front) .

Practically , all solutes can be adsorbed on a microporous solid surface or be partitioned between two immiscible liquids, but, there are some thermodynamic quantities, generally used in physical chemistry , are coupled with the empirical parameters of retention as in the form of 'semi-empirical models of chromatography' .

Snyder (27,28) and Soczewinski (29) established a semi-empirical model in late 1960's to get relationship between the retention coefficient

(R_F) and the thermodynamic adsorption coefficient as ,

$$R_F = \frac{1}{K_{th} \cdot (Va\ Wa) / Vm}$$

Where , Vm is total volume of mobile phase ; Va is the volume of adsorbed mobile phase per unit mass of adsorbent and Wa is the considered mass of adsorbent. The thermodynamic adsorption coefficient (K_{th}) was defined by Scott and Kucera (30,31) with the help of following equation ,

$$K_{th} = \frac{Fp'\ f_1(T)\ Cp' + Fd'\ f_2(T)\ Cd'}{Fp\ f_3(T)\ Cp + Fd\ f_4(T)\ Cd}$$

Where , Fp' and Fd' are polar and dispersive forces respectively between the solute molecules and the stationary phase ; Fp and Fd are polar and dispersive forces respectively between solute molecules and the mobile phase ; Cp' , Cp and Cd are the concentrations of polar moieties and dispersive moieties in the stationary and mobile phases respectively. Cd is proportional to the density of Dispersive medium which can be expressed as concentration in terms of the mass per unit volume . Cd = A.d , where , A is a constant.

Martin and Synge (8,32) established the Martin-Synge model of partition chromatography to ascribe thermodynamic meaning to the retention parameter (R_F). According to this model, R_F value can be viewed as ,

$$R_F = \frac{1}{1 + K\ (Vm / Vs)}$$

Where, Vm and Vs are the volumes of mobile and stationary phases respectively .

In order to develop a quantitative measure the solvent's relative ability

to intermolecularly interact with the solutes as proton acceptors , proton donors and strong dipoles , Snyder (33,34) established a new model coupling the solvent's polarity index (P') with solubility constants (Kg") of the selected test solutes ; ethanol (a model proton donor) , dioxan (a model proton acceptor) and nitromethane (a model strong dipole). The main relationship of this approach is ,

$$P' = \log (Kg")_e + \log (Kg")_d + \log (Kg")_n$$

where, $(Kg")_e$, $(Kg")_d$ and $(Kg")_n$ are measures of the excess retention of given solute (i.e. ethanol, dioxane and nitromethane respectively) relative to an n- alkane of equivalent molar volume .

1.8 BASIC STEPS OF TLC ANALYSIS

The thin layer chromatographic analysis includes the identification, separation and quantitative determination of a substance involving the following basic steps :

(I) SAMPLE PREPARATION

Sample preparation procedures for TLC are similar to those for other chromatographic techniques . The sample solutions to be analysed must be sufficiently pure and concentrated so that the analyte can be easily detected and separated as a discrete , compact spot /zone .If the substance is present at low level in a complex sample ,solvent extraction ,purification and concentration procedures usually precede TLC .Preparative TLC may also be used for the same purpose .An informative chapter on sample preparation for TLC was written by J. Sherma (18) . A. Mohammad listed some solvent systems used to prepare the samples of inorganics and organometallics (35).

For the preparation of metal ion solutions , their nitrates, chlorides or sulfates of analytical reagent grade are dissolved in double distilled water, 0.1M HNO_3 or 0.1M HCl to a final metal ion concentration of 0.05-0.2M. Anion standards are solutions prepared from sodium , potassium or ammonium salts of the corresponding acids using distilled water, dilute acids or alkalies . The metal complexes are generally taken as freshly prepared solutions in ethanol ,acetone ,chloroform or distilled water . The complexes were occasionally dissolved in the corresponding solvent system being used as mobile phase .

(II) TLC PLATE PREPARATION

The available procedures for the manual preparation of thin layers from sorbents (manufactured specially for TLC) include spreading ,dipping ,pouring or spraying the slurry on a glass or plastic plate . Of these procedures , the spreading one is the most common and several types of TLC applicators are available for this purpose .Manually prepared layers should have uniform , bright and translucent appearance when viewed in incident and transmitted light and the layer should also be sufficiently bound to the supporting sheet. Standard TLC plates are 20×20 or 20×10 cm^2 size and the thickness of dried layer for analytical purposes is kept to 0.1-0.3 mm .The most common layer thickness in use at present is 0.25 mm .

There has been a tremendous improvement in the technology of layer materials since 1956 when silica gel with standardized and reproducible properties became available for hand made thin layers primarily due to Stahl's constant efforts. A large number of layer materials have been used as the stationary phase in inorganic TLC but silica gel , as usual has been the

much favoured layer material . The layer materials used so far in inorganic TLC / HPTLC may be categorized as under ;

(a) Non Surface Modified Layers : These are layers prepared from silica gel G / H, alumina , cellulose , polyacrylonitrile (PAN), kieselguhr etc. .

(b) Chemically Modified and Bonded Layers : This group of layers include lipophilic C_{18} bonded silica gel , aminopropyl silica gel , octadecyl silica gel , ECTEOLA cellulose ,carboxymethyl cellulose , aminobenzyl cellulose , diethyl -(2-hydroxypropyl) aminoethyl cellulose , fixion 50×8 etc. .

(c) Inorganic Ion - Exchange Layers : Stannic silicate ,zirconium phosphoantimonate, zinc ferrocyanide , Stannic sulfosalicylate and binder free zirconium antimonate in H^+ form have been used as cation exchange layer materials whereas hydrous antimony (V) oxide has been the most commonly used anion exchanger .

(d) Impregnated Layers : For the preparation of impregnated layers generally , inorganic salts , acids , chlorobenzene , amines , dimethylsulfoxide (DMSO), tributylamine(TBA) , tributylphosphate (TBP) , EDTA, mixture of alizarin red S and aliquat 336 are used as impregnantes in the inorganic TLC .

(e) Mixed Layers : The binary layers that have been used in inorganic TLC include silica gel + microcrystalline cellulose , silica gel + ion-exchange gels , microcrystalline cellulose + modified silica gel H , silica gel + alumina /antimonic acid and kieselguhr + cellulose .

(f) Miscellaneous Layers : These materials include silufol , silufol UV 254, silufol with a layer of silica gel, soil, soil + flyash, polychrome A,

diatomite, chitin, chitosan, kieselguhr, kaolin, and polyamide have also been used as layer materials .

(III) SAMPLE APPLICATION

Sample application is a critical step for obtaining good resolution and quantification in TLC. Manual spotting methods, which involve the use of a variety of sample applicators are most frequently employed in quantitative TLC.

The simplest technique to apply samples spotwise is to use a fixed volume pipette of 100 nL and 200 nL that are available in the form of platinum-iridium capillaries melted in a glass holder. Larger volumes can be applied with fixed volume glass capillaries available for volumes of 500 nL and greater. As an alternative to capillary pipettes, a micrometer controlled syringe can be used for the same purpose. One aspect of the syringe type dosage device is its selectable volume. The nanoapplicator (Nanomat) is an example of micrometer controlled syringe which has a dynamic volume range of 50-230 nL.

An advantageous alternative to sample application as spots is the application of sample in the form of narrow band or streak. This provides the highest resolution attainable with a particular TLC system (36). The Linomate, an applicator, allows sample application in narrow bands by a spray-on technique. By employing this technique, the gain in precision of quantitative TLC analysis can be expected to be in the range of 30 %. With the aid of this device , sample volumes ranging from 2-100 μL can be applied onto HPTLC plate . A valuable aid for sample application in large volumes of very dilute samples is the concentrating zone (Fig. 2.2) which

consists of a chromatographically inactive adsorbent (Kieselguhr) . The substances to be separated are concentrated to a small band at the interface between concentrating zone and the chromatographically active adsorbent (Silica) . Another method of sample concentration is a short pre-elution (few mm) with a solvent , in which all substances have higher R_F values .

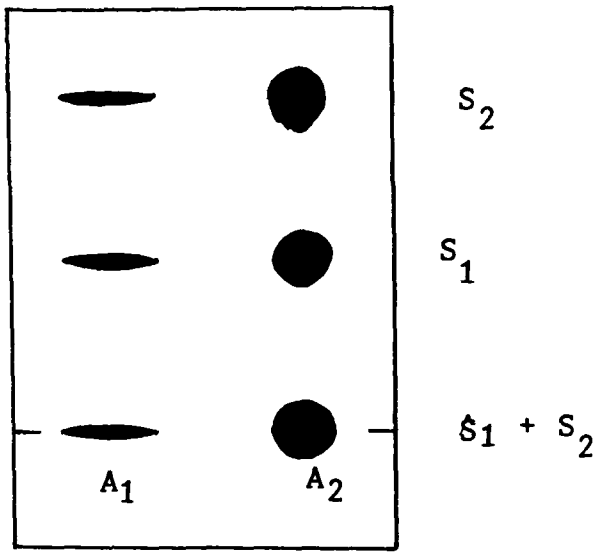


Figure 1.2: Bandwise (A_1) and spotwise (A_2) application of sample (mixture of substances S_1 and S_2).

The automatic TLC sampler III' is the latest version of this kind. It applies sample automatically from a rack of sample vials. The samples are desponded from a steel capillary, spotwise by contact transfer or bandwise by spray-on technique . The capillary moves at a lateral speed of 25 cm/s. The sample dispensing speed is selectable between 10 and 1000 nL/s . The application module is controlled by an IBM-compatible PC , normally the same computer that controls densitometric evaluation of chromatogram .

After sample application , the solvent of the sample is allowed to evaporate completely before the start of development of TLC plate . It can be

achieved either allowing the spot to air dry at room temperature or by blowing the cold or hot air .

(IV) DEVELOPMENT OF CHROMATOGRAM

The movement of mobile phase through sorbent bed is known as its development . In TLC, various factors i.e mobile phase compostion , temperature , humidity and chamber saturation strongly affect the retention of analyte. Numerous modes are available for the development of chromatogram , some are discussed below :

***(a) Ascending*:** This is the most frequently used technique for linear development. Following sample application and drying of the zone , TLC plate is placed in a pre-saturated jar in such a manner that the mobile solvent phase must be below the point of application of the sample .The mobile phase is then allowed to ascend by capillary action , usually to a distance of 10-15 Cm above the origin on 20×20 Cm TLC plate and 3-7 Cm on smaller HPTLC plates .

Covered glass tank in which the TLC plate is placed for development , is known as chamber . Various chambers i.e N-chamber, S-chamber, Twin trough chamber (an N-chamber modified with an inverted V-shaped ridge at the bottom dividing the tank into two sections) are available. A computer controlled automatic developing chamber has been designed to provide programmable , reproducible linear ascending development without operator's attention .

***(b) Descending*:** Downward movement of mobile phase occurs in this type of development .For descending development, the mobile phase must be fed to the top of a vertical inclined plate through a wick arrangment .

This technique has no significant advantages over ascending one and is rarely used for the thin-layer gel filtration chromatography (TLG) for proteins, peptides and nucleic acids . By employing this mode , the development time can be reduced slightly as the capillary forces and gravitational forces function in the same direction .

(c) Horizontal **:** In this type of development, a continuous supply of solvent must be transferred from a capillary arrangment. Develpment can be carried out from one as well as from both sides .The developing solvent held in narrow troughs , is carried to the layer through capillary slits formed between the trough walls and glass slides .The chamber is covered with glass plate during pre-equilibration and development can be operated in N-type or S-type configurations .The horizontal developing chamber permits simultaneous development from opposite edges to the middle of sixty sample spots on 20×20 cm HPTLC plate or thirty samples on a 10×10 cm plate.

(d) Two- Dimensional **:** In fact, two-dimensional development is a techinque but not a mode of chromatogram development for which, the ascending mode is adopted for the full length of the plate to achieve maximum resolution . The plate is air dried, rotated at 90^0 angle and redeveloped usually with a second solvent having a diverse , complementary selectivity. Resolution (spot capacity) in 2-D TLC is greatly improved as compared to 1-D TLC because sample components are resolved over the entire area of the plate. Poole et al. (37) have reviewed methods for combining different retention mechanisms in 2-D TLC .

(e) Radial **:** For radial or circular development, the mobile phase is slowly applied to the center of ring of spots in the middle of a horizontal TLC plate.

Sample components move outward in the form of concentric arcs under the influence of a negative solvent gradient that causes the rear of each zone to move relatively faster than the front. The relation between linear and circular migration is given (38,39) as ;

$$R_{F\,(linear)} = R_F^{\,2}\,(circular)$$

Circular chromatography can be carried out with forced flow as well as under capillary action flow of mobile phase . No special plate preparation is required for off-line radial OPLC or RPC , but for on-line radial OPLC , a sector must be isolated by scraping the layer and then impregnated with solvent(40) .

In addition to above mentioned modes of chromatogram development, multiple (41) , continuous (42-45) , gradient (46,47) and anti- circular (48) development techniques are also available .

MOBILE PHASE:

In liquid chromatography including TLC, the mobile phase exerts a decisive influence on the separation . Various optimization schemes (Windows diagram , Overlapping resolution maps , the Simplex method and PRISMA model) are proposed for normal phase and reversed phase TLC . A large number of mobile phases have been reported , of them some are enlisted below .

(a) Organic Solvents **:** The single component mobile phase comprising of acetone , acetonitrile , benzene , carbon tetrachloride , chloroform , dioxane, ethanol, ethylacetate, methanol , o-xylene , petroleum ether , toluene, n- octanol, n-nonane , cyclohexanes and binary/ternary mixtures of alcohols , amines , ketones , phenols , haloalkanes have been used .

(b) Inorganic Solvents : Being non- toxic and non-volatile , solvent systems of this group have been widely used in TLC of inorganics and organometallics . This group includes the solution of mineral acids , alkalies and inorganic salts prepared in double distilled water or water- methanol mixture .

(c) Mixed Solvents : Mixtures of two or more different solvents, most of which have either a base (NaOH ,NH_4OH , R-NH_3) or an acid (mineral or carboxylic) as a component , are used to develop the TLC plate.

(d) Complexing Solvents: Solutions of surfactants (SDS ,CTAB ,or Triton X-100) , buffered EDTA and DMSO have been used as mobile phase in the TLC of inorganics .

(V) DETECTION AND IDENTIFICATION

After development , TLC plate is removed from developing chamber , dried at room temperature and then activated to make it free from mobile phase .The resolved analyte (s) on plate are detected by their original color, natural fluorescence , quenching of fluorescence or as colored zones produced as a result of chemical reaction of analyte with the detection reagent. The following detection procedures are available for the visualization of spots on TLC plate .

(a) Self Detection : The analytes which are colored or produce colored complexes with stationary / mobile phase , may be viewed in daylight without any treatment . This procedure has limited applicability and is not suitable for the vizualization of trace amounts of an analyte on TLC plate .

(b) Chromogenic Detection :When a complex (colored) forming reagent is applied onto the TLC plate either by spraying or dipping the plate , The

analyte is visualized on the basis of colored spot and this detection procedure is termed as chromogenic detection . Various types of chromogenic reagents are available for the visualization of inorganics ,organics and organometallics (49,50) .

(c) UV Detection **:** Analytes with native fluorescence are viewed as bright zones on a dark background under UV light .For this purpose , shortwave (254 nm) and longwave (366nm) UV lamps are available for use in a dark room . Viewing cabinets incorporating these lamps are also available for inspecting chromatograms under UV light in an undarkened room (51) .

Compounds that absorb UV radiation on an "F-layer" containing a phosphor or fluorescent indicator (often zinc silicate) , when irradiated with 254nm UV light , absorbing compounds quench the uniform layer fluorescence and detected as dark violet spots on bright background .

(d) Flame Ionization Detection **:** A novel detection method for TLC involves the use of a FID in conjunction with sorbent coated rods (52,53) . The coated rods are developed in a saturated N-chamber , dried and advanced automatically at a constant speed through the hydrogen flame of the FID . The separated analytes on the rod are ionized by the flame and the ions generate an electric current , proportional to the amount of substance entering the flame . Detection procedures for a number of compounds , are well documented by J.Sherma (54)

(e) Enzymatic Detection **:** Enzymatic reactions can be monitored on a TLC plate and the end product can be detected . Many pesticides inactivate or inhibit enzymes associated with animal nervous tissue . The presence of minute amounts of such cidal substances may interfere with the indoxyl

acetate substrate reaction, producing colorless spots on background (55,56).

(f) Biological Detection : Biological test procedures are useful for the specific detection of compounds with a certain physiological activity . As a recent example, an agar overlay assay using *candida abicans* as the indicator organism was devised for the detection and activity-guided fractionation of antifungal compounds by TLC . Inhibition of fungal growth was assessed with thimole blue . The technique was compatible with diol and C_{18}plates (57) .

(VI) SEPARATION

When two or more analytes have differential migration with the same chromatographic system , they are mixed thoroughly . The mixture is loaded onto the TLC plate and chromatographed . The separated components of mixture are detected and their R_F values are recorded. Following are some basic requirments for a good separation ,

(a) Each spot should be compact (R_L-R_T < 0.3) .

(b)The difference in R_F values of two adjacent spots should be atleast 0.1 .

(c) No complex formation should be taken place between / among separable species .

(d) Chromatography of individuals and the mixture should be performed in identical manner .

(VII) QUANTITATION

When the amount of an analyte is determined and the method gives an accuracy and reproducibility in the range of 10-30 % , the procedure is considered as semi-quantitative analysis . For more accurate results , method termed as quantitative analysis is performed which gives relatives standard

deviation between 3 and 10% . Following procedures are available for semi-quantitative (a,b) and quantitative (c.d) analyses .

(a) Visual Comparison : The simplest type of *in-situ* quantitative TLC is based on visual comparison of colored , fluorescent or quenched spots . This method is quick and requires no equipment . Comparison is easier on TLC plates coated with adsorbents of wider range of particle's size . Following two characteristics of analyte spot are useful for semi-quantitative determination .

When total forces (polar and dispersive) of stationary phase acting on analyte are highly greater than that of mobile phase , the substance is strongly adsorbed and the intensity of spot increases instead of its size , with increasing amount of substance loaded on TLC plate .Under such conditions, the amount of the unknown sample is determined by matching the intensity of its spot with those of standards .

When forces of stationary and mobile phases acting on analyte are nearly equal, the size of spot increases with increasing loading amount of analyte and the spot's size of unknown amount is compared visually with that of standards .

(b) Spot-Area Measurment : The methods based on the measurement of spot-area can reduce the error of quantification to the range 5-15 % . The spot-area can be measured by anyone of the following methods ;

In graphical method , the size and shape of the spot is copied from TLC plate onto the graph paper and the number of squares inside the spot's periphery are counted . This method has highest probability of error be cause an square is counted only when its half or more portion is inside the spot-

area otherwise the square is left , as shown in figure 1.3 .

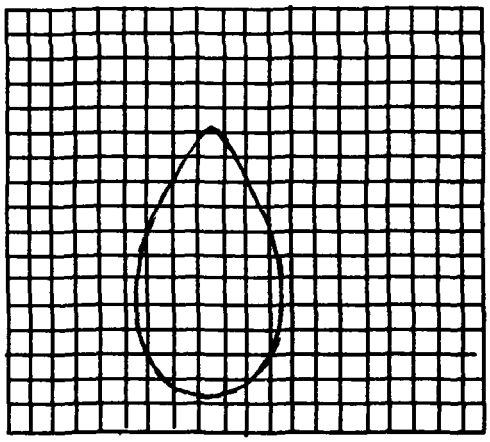


Figure 1.3 : Spot- area Measurement by Graphical Method (Area =43 mm^2).

In area-weight method , size of spot is copied onto the filter sheet followed by cutting and weighing of the filter paper corresponding to the spot-area . The area-weight (weight of filter paper covering the spot-area) is plotted against the loading amount of analyte species to sketch the calibration curve.

Algebraic method avoids the plotting of calibration curves and is based on the three analyses (a sample , diluted sample and a standard) with the same chromatographic system . The calculation of unknown amount is made on the basis of following equation .

$$\log W = \log Ws + \left[\frac{\sqrt{A} - \sqrt{As}}{\sqrt{Ad} - \sqrt{A}} \right] \log D$$

Where W and Ws are weights of solute in sample (before dilution) and standard respectively ; D is the dilution limit ; A , Ad and As are spot-areas of solute in sample (before dilution), diluted sample and in standard respectively .

The RSDs for analyses using this method have been reported between 3 and 10% . Linear relationships have been found between spot-areas and log weight (58) as well as between square root of spot-area and log weight (59) . The exact relationship yielding a linear plot has been found to depend on the layer thickness, nature of the solute and the detection reagent(60). As an attempt to standardize the quantitative TLC , Mohammad and Fatima (61,62) , Nanda and Devi (63) and Mlodzikowski (64) have established a linear relationship between the spot-size and the amount of substance .

(c)In-Situ Densitometry : It is the most preferred technique for quantitative analysis where the determination of the sample is performed by *in-situ* measurement of absorbance or fluorescence of a separated zone using an optical beam in the form of a rectangular slit . A schematic diagram of a single beam scanner arranged for measurement of absorption in the reflectance mode is shown in Figure 1.4.

The purpose of scanner is to convert the spot on a layer into a chromatogram consisting of a series of peaks similar in appearance to GC or HPLC. Halogen or tungsten lamps are used to provide light of 400-800 nm range (visible absorption), deuterium lamp for scanning directly or as quenched zones on 'F' layers in the 190-400 nm range (UV region) while for fluorescence scanning , a high intensity xenon or mercury lamp is used as the source . Filters or monochromators (prism or grating) are employed for wavelength selection and a photomultiplier (photodiode) for signal measurement. The plate , mounted on a movable stage is scanned with a fixed beam of monochromatic light . Reflected or transmitted light is measured with

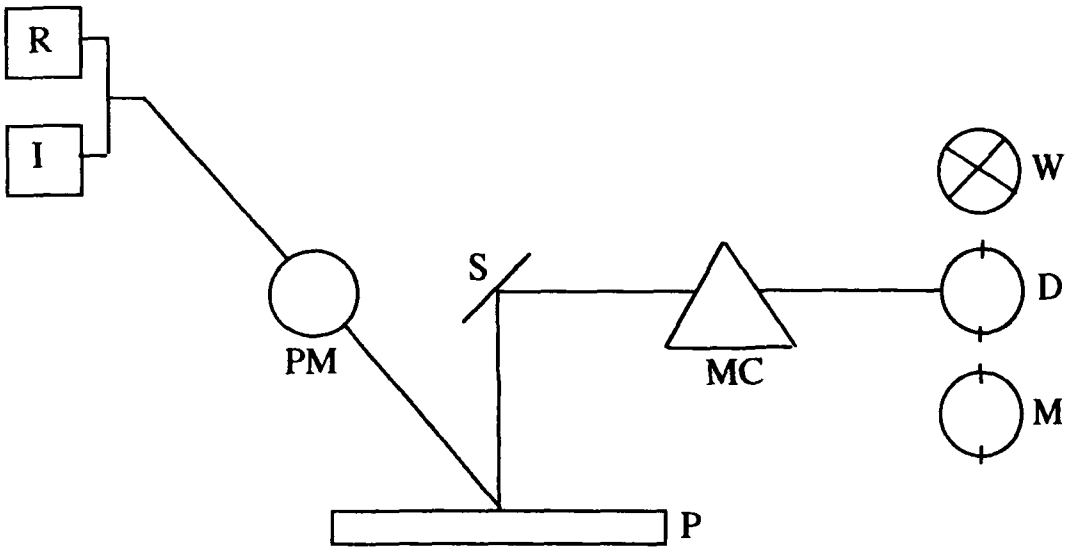


Figure1.4: Schematic Diagram of Optical Path for Densitometric Absorption Scanning .W,Tungsten lamp; D, Deuterium lamp; M , Mercury lamp; MC , Monochromator; S, Mirror; P, Plate; PM , Photomultiplier detector; R, Recorder; and I is Integrator .

either single beam , double beam or single beam - dual wavelength optical systems. The modern scanner with a computer controlled motordriven monochromator, allows automatic recording of *in-situ* absorption with fluorescence excitation spectra. The method based on fluorescence has been preferred over absorption for quantitative densitometric analysis because of the higher sensitivity , better selectivity, wider linear range of calibration curve (peak height Vs concentration).

Transmission or reflectance scanning can also be used for photometric evaluation of substances . TLC quantification by *in - situ* densitometry (65,66) and sources of error in densitometric evaluation (67) have been reviewed . The relative standard deviation of *in-situ* densitometry can be maintained below 2%. It is a reliable analytical technique for quanitfication (68) .

(d) Zone - Elution and Photometry : In 1958, Kirchner et al . (69) first reported the application of TLC to the quantification in combination with spectrophotometric measurements , As a result of achievements made in this field, reflectance photometry , emission spectroscopy , A AS etc. have been combined with TLC for the quantitative analysis .

The TLC -spectrophotometry involves drying the layer , locating the resolved zones , scraping off the separated zones of sample and standards and elution of the analyte from the layer material with a suitable solvent . The eluted substances are concentrated and analyzed by any Independent microanalytical method . Spectrophotometry has been the most widely used technique for the quantification of eluted inorganic species (70-75) .

REFERENCES

1. H.G. Cassidy , in *Fundamentals of Chromatography* ,2nd edn. , Interscience Publishers , New York (1957) 2 .
2. A.I.M. Keulemans , in *Gas Chromatography* ,2nd edn., Reinhold Publishing Corp. , New York (1959) 2 .
3. M.Tswett , *Ber. Deut. Botan. Ges.* 24 (1906) 235 .
4. M.Tswett , *Ber. Deut. Botan. Ges.* 24 (1906) 316 .
5. R.Kuhn and E.Lederer , *Ber.* 64 (1931) 1349 .
6. R.Kuhn , A. Winterstein and E. Lederer, *Hoppe-seyler's Z. Physiol . Chem.* 197 (1931) 141 .
7. A.J.P. Martin and R.L. M. Synge , *Biochem. J.*35 (1941) 91.
8. A.J.P. Martin and R.L. M. Synge , *Biochem. J.* 35 (1941) 1358.
9. K. Dorfner, in *Thin Layer Chromatography-A Laboratory Handbook*, 2nd edn., E. Stahl (Ed.) , Springer-Verlag , New York (1969) 44 .
10. N. Pelick , H.R. Bolliger, and H. Mangold, in *Advances in Chroma tography* , J.C. Giddings and R.A. Keller (Eds.) , Marcel Dekker , New York (1966) 85 .
11. E. Stahl, in *Thin Layer Chromatography* , 2nd edn. , E.Stahl (Ed) , Springer-Verlag , Berlin (1969) 1 .
12. E. Heftmann, in *Chromatography- A Laboratory Handbook of Chromatographic and Electrophoretic Methods* , 3rd edn. , Van Nostrand Reinhold, New York (1975) 1 .
13. J.G. Kirchner , J. Chromatogr. Sci . 13(1975) 558.
14. J.G. Kirchner, in *Thin Layer Chromatograpy*, 2nd edn., Wiley-Interscience, New York (1978).

15. J.G. Kirchner, in *Thin Layer Chromatography-Quantitative Environ mental and Clinical Applications* , J.C. Touchstone and D. Rogers (Eds.) , Wiley-Interscience , New York (1980) 1.

16. H. Jork and H. Wimmer, in *TLC Report : A Collection of Papers* , GIT Verlag , Darmstadt (1986) .

17. U. Wintermeyer, in *The Root of Chromatography : Historical Outline of the Begining of Thin Layer Chromatography* , GIT Verlag , Darmstadt (1989) .

18. J.Sherma, in *Handbook of Thin Layer Chromatography* , 2nd edn. , J.Sherma and B. Fried (Eds.) , Marcel Dekker , Inc., USA (1996) 3 .

19. N.A. Izmailov and M.S. Schraiber , *Famatsiya* 3(1938) 1 , .

20. J.E. Meinhard and N.F. Hall, *Anal. Chem.* 21 (1949) 185 .

21. J.G. Kirchner , J.M. Miller and G.J. Keller , *Anal . Chem.* 23 (1951) 420 .

22. E. Stahl , *Pharmazi* (1956) 633 .

23. E.Stahl , *Chem . Z.*, 82 (1958) 323 .

24. E.P. Przybylowicz , W.J. Staudenmayer , E. S. Perry , A.D. Baitsholts and T. N. Tischer , *J. Chromatogr* . 20 (1965) 506 .

25. A.Zlatkis and R.E.Kaiser, in *High Performance Thin - Layer Chromatrography*, Elsevier, New York (1976) .

26. E. Tyihak , E. Mincsovics and H. Kalasz , *J. Chromatogr.* 174(1979) 75.

27. L.R. Snyder , in *Principles of Adsorption Chromatography* , Marcel Dekker , New York (1968) .

28. L.R. Snyder , *Anal . Chem .* 46 (1974) 1384 .

29. E. Soczewinski , *Anal . Chem .* 41 (1969) 179 .

30. R.P.W. Scott and P. Kucera , *J. Chromatogr .* 112 (1975) 425 .

31. R.P.W. Scott , *J. Chromatogr .* 122 (1976) 35 .

32. A.J.P. Martin , *Biochem . J.* 50 (1952) 679 .

33. L.R.Snyder , *J. Chromatogr .* 92(1974) 223 .

34. L.R.Snyder , *J. Chromatogr . Sci .* 16(1978) 223 .

35. A. Mohammad , in *Handbook of Thin Layer Chromatography* , 2nd edn . , J.Sherma and B. Fried (Eds.) , Marcel Dekker , Inc . , U.S.A. (1996) 507.

36. D.E. Jaenchen, *Proc. Intl . Symp . Instrumental HPTLC* , R.E. Kaiser (Ed.) , Bad Duerkheim , F.G.R. (1985) 71 .

37. C.F. Poole , S.K. Poole , W.P.N. Fernando , T.A. Dean , H.D. Ahmad and J.A. Berndt , *J. Planar Chromatogr.- Mod. TLC* 2(1989) 336.

38. A. Po. , Li Wan and W.J. Irwin , *J. High Resolut . Chromatogr. Chromatogr . Commun .* 2 (1979) 623 .

39. R.E. Kaiser , in *High Performance TLC* , A. Zlatkis and R. E. Kaiser (Eds.) , Elservier , New York (1977) 15 .

40 . Sz. Nyiredy , in *Chromatography* , 5th edn ., E. Heftmann (Ed.) , Elsevier , Amsterdam (1992) 109 .

41. J.A. Perry ,T.H. Jupille and L.H. Glunz , *Anal . Chem .* 47 (1975) 65A.

42. R.E. Tecklenburg and D. Nurok , *Chromatographia* 18 (1984) 249.

43. R.E. Tecklenburg ,G.H. Fricke and D.Nurok , *J.Chromatogr .* 290 (1984) 75 .

44. D.Nurok . R.E. Tecklenburg and B.L.Maidak . *Anal . Chem .* 56 (1984) 293 .

45. D.Nurok . R.M. Becker and K.A. Sassic, *Anal . Chem .* 54 (1982) 1955 .

46. N. Grinberg , in *Modern Thin Layer Chromatography* , N. Grinberg (Ed.) , Marcel Dekker , New York (1990) 368.

47. W.Golkiewicz , in *Handbook of Thin Layer Chromatography* , J.Sherma and B. Fried (Eds.) , Marcel Dekker , New York . (1991) 135 .

48. R.E.Kaiser , *J.High Resolut. Chromatogr.Chromatogr .Commun .* (1978) 164 .

49. A. Mohammad and K.G.Varshney , in *Handbook of Thin Layer Chro matography* , J.Sherma and B.Fried (Eds.) , Marcel Dekker , New York (1991) 463 .

50. A. Mohammad . N.Fatima , J.Ahmad and M.A.M.Khan , *J.Chromatogr.* 642 (1993) 445 .

51. M.F. Gonnord and A.M. Siouffi ,*J. Planar Chromatogr-Mod. TLC* 3 (1990) 206 .

52. M.Ranny , in *Thin Layer Chromatography with Flame Ionization Detection* , D.Reidel Publishing Co. , Dodrecht (1987) .

53. K.D.Mukherjee , in *Handbook of Thin Layer Chromatography* , J.Sherma and B.Fried (Eds.) ,Marcel Dekker , New York (1991) 339 .

54. J.Sherma . *Thin Layer Chromatographic Techniques and Applications* J. Sherma (Ed.) , Marcel Dekker , New York (1994) 146 .

55. J.C.Touchstone , in *Practice of Thin Layer Chromatography* , 3rd edn. , Wiley Interscience , New York (1992) 311 .

56 . C.E. Mendoza , *Residue Rev* . 43 (1972) 105 .

57. L.Rahalison ,M . Hamburger , K. Hostettmann , M.Monod and E.Frank, *Phytochem.Anal* .2 (1991) 199 .

58. M.Brenner and A. Niederwiesor , *Experimentia* 16 (1960) 378 .

59. S.J. Purdy and E.V.Truter , *Analyst* 87 (1962) 802 .

60. N.Nybom , *J. Chromatogr.* 28(1967) 447 .

61. N. Fatima and A. Mohammad , *Sep. Sci. Technol.* 19 (1984) 429 .

62. A.Mohammad and N. Fatima , *Chromatographia.* 22 (1986) 109 .

63. N.V.K. Nanda and Y.P.Devi , *J.Assoc . Off. Anal . Chem.* 64 (1981) 729.

64. J.Maslowska and Z.Mlodzikowski , *Chem . Anal.* 31 (1986) 193 .

65. C.F.Poole and S.K. Poole , *J.Chromatogr.* 492 (1990) 539 .

66. V.A.Pollak and J.Schulze-Clewing , *J. Planar Chromatogr . - Mod . TLC* 3 (1990) 104 .

67. V.A. Pollak , *Adv. Chromatogr.* 30 (1989) 201.

68. C.F. Poole and S. Khatib , in *Quantitative Analysis using Chromato graphic Technique* , E. Katz (Ed.) ,Wiley , New York (1987) 193 .

69. J.G. Kirchner , J.M.Miller and R.G.Rice , *J. Agric .Food Chem .* 2(1954) 1031.

70. M.P. Volynets , R.F. Gurava and T.V.Dubrova , *Zh . Anal . Khim .* 46 (1991) 1595 .

71. R..Shukla and A.Kumar, *Zh. Anal. Khim.* 46 (1991) 150 .

72. A.Asolkar , A. Kumar , P.Pandey and R.Bhardwaj , *J.Liq. Chromatogr.* 15 (1992)1689 .

73. E.I.Morosanova , L.S.Selivestrova and Yu. A.Zolotov , *Zh. Anal. Khim.* 48 (1993) 617 .

74. A. Mohammad and M.A.M. Khan , *J. Chromatogr . Sci.* 33 (1995) 531 .

75. M.Ajmal , A. Mohammad , N. Fatima and A.H.Khan , *Microchem . J.* 39 (1989) 361 .

CHAPTER -II

THIN - LAYER CHROMATOGRAPHY OF INORGANIC IONS SINCE 1980

2.1 INTRODUCTION

Thin-layer chromatography (TLC) has always been recognized as a practical and effective technique for purifying materials before their analysis with sophisticated instruments . It was one of the chromatographic techniques applied for the routine analysis in preparative chemical laboratories.

In recent years , TLC has grown much in status and has experienced a dramatic surge due to its simplicity, versatility, and low cost. It is now being used as an efficient and rapid detection technique . The combination of speed , sensitivity and simplicity with resolution which exceeds that of low pressure column Chromatography and paper chromatography ensures continued interest in TLC With optimization of techniques and materials, TLC can be applied for the quantitation of various compounds present in environmental and geological matrics .

The work on TLC of inorganics published upto the end of 1972 has admirably reviewed by Brinkman et al. (1) and that appeared during 1972-1980 has been presented by Kuroda and Volynet (2). The latest work on TLC of inorganics and organometallics covering the period 1978-1995 has been described in chapters (3,4) of the *Handbook of Thin-layer chromatography* edited by J.Sherma and B.Fried .

The majority of work on inorganics belongs to cations as compared to anions (Figure 2.1) .Transition and inner transition metals have been the popular choice because of their technological importance whereas inorganic anion TLC was performed on halides, helates , cyanides,S-,N-, P-containing and multiple anions .

In a recent report (5) .J.Sherma has excellently discussed the techniques and instrumentation of HPTLC . Regarding publications on its applications. the interested readers are advised to consult the reviews (6-11) and books (12-20) . The information regarding general features . comparative performance of stationary and mobile phases . detection methods , development modes and data processing of inorganic TLC can be found in various review articles (21-35) .Besides inorganic TLC , some important applications of TLC alongwith its theory, principle and instrumentation have been well presented by E.Heftmann (36) .

The purpose of this chapter is to present a summary of work done on TLC of inorganic ions during 1980-1996 . Total number of papers , published in a journal during this period , is plotted against the journal (Figure 2.2) . Top ten journals are ;

1. *J.Planar Chromatogr.* (J_1)
2. *J.Liq. Chromatogr.* (J_2)
3. *Chromatographia* (J_3)
4. *Fresenius J.Anal. Chem.* (J_4)
5. *J. Chromatogr.* (J_5)
6. *Zh. Anal. Khim.* (J_6)
7. *Fenxi Huaxue.* (J_7)
8. *J. High Resolut. Chromatogr. Chromatogr. Commun.* (J_8)
9. *J.Chromatogr. Sci.* (J_9)
10. *Mikrochim. Acta.* (J_{10}) .

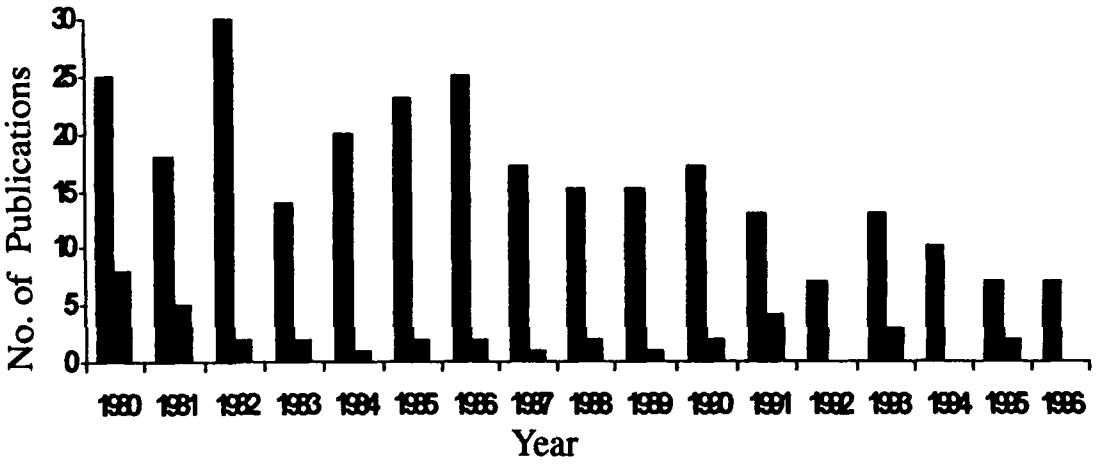


Figure 2.1 : Relative Study of Work Done on the TLC of Inorganic of Anions ▬ and Cations ▭

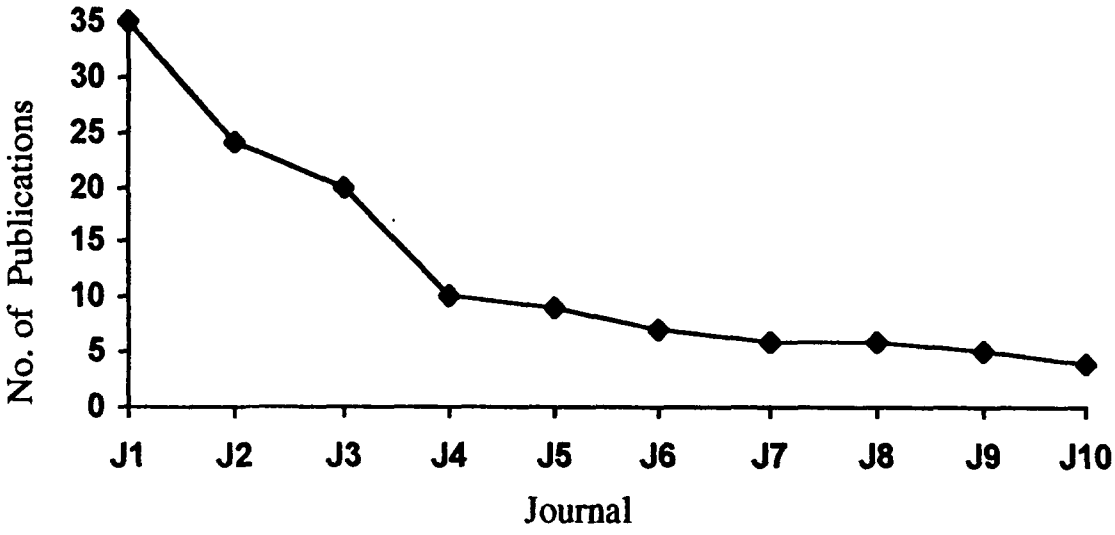


Figure 2.2 : Top Ten Journals Regarding the Number of Publications on TLC of Inorganic Ions (1980-1996)

Table 2.1 ; Thin-Layer Chromatography of Inorganic Ions.

Ion / metal	S.P.	M.P.	Comment	Ref.
Ba, Sr,La,Ce, Pr,Nd,Pm,Sm,Eu, Y,Te,Mo,Zr,U	S_1	M_1	Two-dimensional development; qualitative analysis of lanthanides on 2mm silanized silica gel layers	37
Cu, Zn,Ni	S_2	M_2	Saturated chamber; run 10 cm; a relationship between the ion-exchange constant and experimental R_F values was derived, to enable transfer of the theoretical approaches developed for column ion exchange chromatography to TLC.	38
Forty-nine ions including W,Ha, Tl, Mo, Re, Sn, Pb, Cr, Ni, Cd, Zr, Co, Te, Sc, Sb, Cu, Ag, As, Be, Fe, Mg, Th, Y, Ge	S_3	M_3	Development time 35-45 min; layer thickness 0.25 mm; run 17 cm; qualitative separations .	39
Bi, Th, Zr, Ce, Cr, Al, Ti, W, UO_2 , Sn, Fe, Sb, V	S_4	M_4	TLC-photometric method for separation and determination of micro amounts of Mo(VI) in the presence of interfering cations	40
Eighteen metal ions	S_5	--	Identification and separation of inorganic cation .	41
Cations	S_6	M_5	Qualitative separations.	42
Forty-eight metal ions	S_7	M_6	Qualitative separations.	43
Rare-earth metals	--	M_7	Separation of rare-earth metals in ores (monazite) rockes, pure $Ce(NO_3)_3$ and irradiated nuclear fuels, separation of rare-earths from uranium .	44
V, Th, As, Pb, Zr, Se,	S_8	--	A correlation between R_F values of metal ions on impregnated layers and the stability constant is discussed .	45
Twenty-two cations	S_4, S_9	--	Qualitative analysis	46

Ion / metal	S.P.	M.P.	Comment	Ref.
Twenty-three metal ions	S_{10}	M_8	Binary and ternary qualitative separations .	47
Inorganic metal ions	S_9	M_9	High loading blurs the separation of cation mixtures .	48
Group I, IIA, IIB, and IIIB, cations	S_{11}	M_{10}	EtOH–$CHCl_3$–HCl and BuOH–$HCONH_2$ solvent and systems give best results .	49
Several metal/ ions	S_{12}	M_{11}	Quantitative separation of microgram quantities of Hg(II) from several other metal ions .	50
Some anions	S_{13}	M_{12}	Ascending technique; run 11 cm; qualitative separations; microgram separation of Cr(VI) from several ores and alloys .	51
$Fe(CN)_6^{3-}$, $Fe(CN)_6^{4-}$, MnO_4^-, $Mo_7O_{24}^{6-}$, $S_2O_8^{2-}$, $S_2O_5^{2-}$, SeO_3^{2-}, SeO_4^{2-}, WO_4^{2-}, $Cr_2O_2^{2-}$, $Cr_2O_7^{2-}$, VO_3^-, BrO_3^-, IO_4^- ClO_2^-, ClO_3^-, ClO_4, NO_2^-, NO_3^-	S_{14}	M_{13}	Paper electrophoresis; qualitative TLC and densitometric determination of anions using diphenylamine as spray reagent; detection of NO_3^- and $Fe(CN)_6^{3-}$ in molasses	52
Cl^- Br^-, NO_3, SCN^-, I^-	S_5	M_{14}	Qualitative Separations	53
Twenty-two anions including CN^-	S_{15}	M_{15}	TLC of 22 anions and 25 cations; detection of CN^- as $Hg(CN)_2$.	54
Polyphosphates	--	--	Determination of condensed phosphates in commercial liquid polyphosphate fertilizers	55
Rh (III) , Ir(III) , Ir (IV)	S_{16}, S_{17}	M_{16}	Separation by anion-exchange TLC	56
Ni, Mn , Co , Cu	S_{18}	M_{17}	Separation on silica gel was poor; addition of ion exchange resin markably improved the resolution .	57

Ion / metal	S.P.	M.P.	Comment	Ref.
Au, Ir(III), Ir(IV),Pt, Pd, Ru, Rh	S_{17},	M_{18}	Qualitative separations	58
Rare-earth metals	S_1	M_{19}	Separation and detection of rare-earth metals in a variety of mixtures	59
Forty metal ions	S_{19}	M_{20}	Qualitative analysis	60
Transition metal ions	S_7	M_{21}	Separation of Fe(III) , Ce(IV), Cu(II), Co(II) , Cr(III) and Ni(II)	61
Rare-earth metals	S_1	M_{22}	Qualitative separations	62
Mo, Y, La,	S_5 , S_{20}	M_{23}	Chromatographic behavior of La, Y and Mo was investigated .	63
Ni, Cu, Hg, Pb, Bi, As, Ce, Tl	S_6	M_{24}	Separations on the basis of differential solubilization of metal chloro complexes .	64
Cr(III), Cr(VI)	S_{21}	M_{25}	Optimum condition for quantitative separation of Cr(III) and Cr(VI) from their binary mixtures .	65
Hg, Cu, Cd, Ag	S_7	M_{26}	Concentration, separation and detection of microgram amounts of Cu, Ag, Hg, and Cd salts in fresh water	66 67
SCN^- , selenocyanate	S_7	M_{27}	Detection and micro-determination of anions by densitometry.	68
I^-, Br^-, Cl^-, SCN^- ClO_3^-,BrO_3^-, IO_3^-, NO_2^- , $S_2O_3^{2-}$,SO_4^{2-}, CrO_4^{2-},PO_4^{3-} , AsO_3^{3-}, AsO_4^{3-}, $Fe(CN)_6^{3-}$, $Fe(CN)_6^{4-}$	S_5	M_{28}	Thin layer stick chromatography .	69
Cd, Bi, Hg, Pb, Pt, Th, As, Ru, Pb, Te, Sc, Ag, Ge, Sb, Zr, W, Re, Sn, Tl, Zn, Mo, Cr, UO_2 .	S_{22}	M_{29}	Ascending technique; run 17 cm ; development time 40-60 min; layer thickness 0.5 mm .	70

Ion / metal	S.P.	M.P.	Comment	Ref.
BrO_3^-	S_5	M_{30}	Detection and recovery of BrO_3^- from flour , dough, and breads by TLC .	71
$Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$	S_7 S_{21}	M_{31}	Quantitative separation and recovery of anion from bleach and fixer solutions.	72
Cl^- ,Br^-	S_5	M_{32}	Detection of Br^- in tissue samples, in the presence of Cl^- , by peak area measurement; results are linear for 0.02-0.5 molar Br^- .	73
Cu, Hg, Zr, Zn, Cr, Y, Gd, Ho, Nd, La, Pr, Ce, Pd, Au, Pt, Co, Ni, Fe, Bi	S_{23}	M_{33}	Spectrophotometric determination of Pd^{2+}, after elution from the TLC plate, using *p*-nitrosodimethylaniline as chromogenic reagent	74
Zr, Ce, W	S_5	M_{34}	Quantitative separation of Zr^{4+} from lanthanum and Ce^{3+}; W^{6+} from Ce^{4+}, Mo^{6+} and vanadyl(V^{2+}).	75
Twenty-five cations	S_6	M_{35}	Development time 15-25 min , TLC on microscope slides .	76
Rh(III), Ir(III) , Ir(VI)	S_{16}, S_{17}	M_{36}	Anion exchange separations .	77
Gold, selenium, tellurium	S_5, S_{24}, S_{25}	M_{37}	Separation , detection and determination of micro amounts of Au(III) . Se(IV), and Te(IV) .	78
Fe, As, Sb, Hg, Sn, Cr,	S_{26}, S_{27}	M_{38}	Separation of different valency states	79
Rare-earth elements	S_{28},	M_{39}	Separation and spectrophotometric determination of rare-earth elements; application to the determination of La, Ce, Pr and Nd in monazite .	80
Ba, Y, La, Ce	S_{29}	M_{40}	Quantitative separation from Sr.	81
Forty-seven metal ions	S_{30}	M_{41}	Quantitative separations and adsorption behavior .	82
Nineteen metal ions	S_5, S_{31}	M_{42}	Detection limits of metal ions on plates was 1.0-7.0 mg .	83
NO_3^-	S_{14}	M_{43}	Densitometric determination of NO_3^-	84
Phosphate, silicate	S_5	M_{44}	Determination of phosphate and silicate as their molybdate complexes .	85

Ion / metal	S.P.	M.P.	Comment	Ref.
PO_4^{3-},$P_2O_7^{4-}$, $P_4O_{10}^{5-}$	S_6	M_{45}	Detection of phosphate on cellulose layer in the presence of molybdenum.	86
Twenty-six anions	S_6	--	Qualitative separations on cellulose coated microscope slides .	87
Pb, Mn, Zn,Cd, Ni, Co,Cu, Fe, Mg, Pd,V,UO_2, Th	S_5, S_{32}	M_{46}	Dibenzoyl methane impregnation offers a method for the removal of iron from silica gel G.	88
Forty-nine inorganic ions	S_{33}	M_{47}	Qualitative separations .	89
Forty-eight metal ions	S_7	M_{48}	Qualitative separations .	90
Lanthanides	--	M_{49}	HPLC and TLC excellently supplement each other in the separation of lanthanides; application to the separation of fission products .	91
Ni, Ca, Cu, Mn	S_{34}	M_{50}	Preparation of stationary phase and its application in inorganic TLC .	92
Rare-earth elements	S_{28}	M_{51}	Separation of La, Ce, Pr, Nd, Sm, Eu, Tb and Ho .	93
Ti, Zr, AsO_4^{3-} , MoO_4^{2-} , Th, Al, Cr, Fe, Ce, VO, Be, Bi, Ni, UO_2 , Co, Cu, Hg, Pb, Mn, Zn, Cd, Sb, Sn	S_4	M_{52}	Ascending technique: conditioning time 10 min ; development time 15-20 min; layer thickness 0.25 mm; plate activation at 120° C for 1 h ; quantitative separation of Zr and Ti from each other and from other interfering ions .	94
Cu, Cd, Zn, Ni, Co, Fe, Pb, Cr, Al Zr, V, Th, Cd, UO_2^{2+} ,Ag, Se, VO^{2+}	S_5, S_{35}	M_{53}	Ascending technique; run 10 cm ; development time 12-15 min ; 0.25 mm layer, semiquantitative determination of nine cations on silica gel impregnated with sodium molybdate .	95
Cl^-, Br^-, I^-, IO_4^-, ClO_4^-, BrO_3^-, SCN^-, MnO_4^-, NO_3^-,OAc^- , CrO_4^{2-}, AsO_3^{3-}, AsO_4^{3-}	S_4	M_{54}	Qualitative separations and identification of anions .	96

Ion / metal	S.P.	M.P.	Comment	Ref.
$Fe(CN)_6^{3-}$, $Fe(CN)_6^{4-}$				
Monofluorophosphate	S_6	M_{55}	Detection of Monofluorophosphate in extracts of toothpastes; cellulose layers .	97
NO_3	S_5	--	Detection of NO_3^- in food and water samples ; fifty five cations or anions do not interfere with detection .	98
Dy, Gd, Nd, La, Pr, Tb, Sm, Th, Ti, Zr, and some rare-earths	S_5 , S_{36}	M_{56}	Ascending technique; run 12 cm; 0.5 mm layer; impregnant concentration (1%) in EtOH–H_2O (70:30); plates dried for 24 h at 40-61° C; development temperature 22-61° C .	99
Pb, Cd, Hg, Te, W, Mn, Ag, Fe, Be, Ni, Mg, Pt, Ga, Cu, Ti, Se, Co, As	S_{37}	M_{57}	Ascending technique; run 17 cm; development time 60-80 min; 0.25 mm layer; qualitative separations .	100
Pb, Mg, Ca, Cd, Zn, Fe, Mn, Hg, As, Th, Fe(II), UO_2 (II), V(V)	S_{38}	M_{58}	The impregnated silica gel plates were activated at 60°-61° for 24 h; run 10 cm .	101
Se, Te, and Au	S_{24}	M_{59}	Plates developed in Stahl chamber .	102
Bi, Au, Mn, Co, Zn, Ti, Cu, Ag, Ni, Pt, Cd, Pb, Hg, Tl(I)	S_{39}	M_{60}	Ascending technique; run 11 cm; development time 2 h; quantitative separation of Bi^{3+} from some ternary and quaternary mixtures of metals .	103
VO^{2+},Fe, Al, Fe^{2+}, UO_2^{2+},Bi, Tl^+, Mo, Cd, Cu, Pb, Zn, Co, Th, Se, W, Zr, Cr	S_{40}	M_{61}	Ascending technique; run 10 cm; development time 15-20 min; butylamine-impregnated silica gel layers were selective for metal ions at lower mobile phase pH values (pH 1-2.5)whereas *t*-butylamine impregnated silica gel layer were more selective at higher mobile phase pH values .	104

Ion / metal	S.P.	M.P.	Comment	Ref.
Rh(VII), Mo(VI), V(V)	S_{24}	M_{62}	Study on the effect of nature and composition of the mobile phase on the chromatographic behavior of Ru, Mo and V .	105
Rare- earth metals	S_5	M_{63}	Continuous separation of rare-earth metals, La, Ce, Pr, Nd; detection of Sm in monazite sand .	106
Fe, Pb, Co, Zn, Cd, Cu	S_{28}	M_{64}	Qualitative separations .	107
High valence metal ions	S_{41}	M_{65}	Separation of binary and ternary mixtures of rare-earths, Ti(IV) Zr(IV), Nb(V), and U(VI) .	108
Alkaline earths and transition metals	S_{42}	M_{66}	Separation of Ca^{2+}- Sr^{2+}, Ca^{2+}-Ba^{2+}, Hg^{2+} from Cu^{2+}, Ni^{2+}, Zn^{2+},Co^{2+} and Mn^{2+}.	109
Noble metals	S_{43}	--	Separation of noble metals from commonly present metals .	110
NO_2^-	S_5	M_{67}	TLC and densitometric determination of nitrite in saliva .	111
Br^-,and I^-	S_{44}	M_{68}	TLC detection of Br^- in the presence of I^- .	112
I^-,Br^-, F^-, IO_3^-, BrO_3^-, NO_2^-	S_7, S_{45}	--	Qualitative separations .	113
Phosphates	S_{46}	M_{69}	Identification and determination of phosphates in dairy products .	114
Co, Cu, Ni	S_{47}	M_{70}	Separation and detection as DEDTC	115
Thirty-six cations Fe^{2+}, Zn, Cd,	S_4	M_{71}	Qualitative analysis of 36 common cations .	116
Ni, Co, Cu, Pb, Hg, Al, Tl^+, W, UO_2,Se, Nb, VO^{2+}	S_7	M_{72}	Most effective concentration range of HCOOH in butanol was 0.5 –2.0 M : the presence of ethylmethyl ketone in the HCOOH-butanol system improves the resolution of the separation .	117
Y, Sm, Nd, Pr, Ce, La, Lu, Yb, Tm, Er, Ho, Dy, Tb, Eu, Gd	S_{48}	M_{73}	Ascending technique; development time 20 min for 10 cm run; 250 mm . layer; plate activation at 70^0 C for 20 min, sample volume 0.5 mL .	118

Ion / metal	S.P.	M.P.	Comment	Ref.
Cu, Fe, Mn	S_9	M_{74}	Separation and detection of Cu, Fe, and Mn in cotton materials (lower limit 20 mg / g material).	119
Ag, Al, As, Au, Ba, Be, Bi, Ca, Ce, Ce^{4+}, Co, Cr, Cu, Dy, Fe, Ga, Ge, Hg^{+}, Hg, Ln, La,Mg,Mn, Mo^{6+}	S_{49}	M_{75}	Qualitative separations on treatment with PEI–cellulose.	120
Al, Ni, Cr, Mn, Co, Zn, Fe	S_{50}	M_{76}	Ascending technique; development time 3 h; loading volume 2mL of acidic extract; determination of micro elements in Polfamik products using standard curves prepared from spot surfaces of separated metal ions on TLC plates.	121
Fe^{2+},Cr^{3+}, Zn^{2+}, Co^{2+},Fe^{3+}, V^{4+}, Ni^{2+}, Cu^{2+}	S_{51}	M_{77}	Simultaneous separations and semiquantitative determination of several transition metals according to the color reaction appearing in the sorption zone of the element on the plate ; semequantitative determination of Ni ,Cu and Cr in waste waters.	122
Ag,Al,Cu,Th, Mo, Zn ,Cd, Pb, Se, Zr, Ni, W, UO_2^{2+},VO^{2+}, Fe , Tl^{+}, Co	S_{52}	M_{78}	Semiquantitative determination of Pb, Ag, and Th by spot area measurement method and spectrophotometric determination of UO_2^{2+} after separation from other metal ions.	123
Twenty-Two metal ions	S_7	M_{79}	Semiquantitative determination of nine cations by spot area measurment method , most effective concentration range of formic acid was 0.1–1.0 M	124
Forty-nine inorganic ions	S_{49}	M_{80}	Ascending technique; run 17 cm; development time 100–120 min. ; qualitative separations.	125
Rare-earths , Cd, Co, Pb, Cu, Zn, U(VI) , Th	--	M_{81}	Qualitative separations.	126

Ion / metal	S.P.	M.P.	Comment	Ref.
Light rare-earth metals	S_{53}	M_{82}	Effective separation of light rare-earths in the presence of heavy rare-earths	127
Light rare-earth metals	S_{54}	M_{83}	Separation in monazite	128
Mn(II), Mn(III),	S_7	M_{84}	Ascending technique ; run 20 cm. layer thickness 250 mm.; optimum conditions were determined for quick quantitative separation of di-and tri-valent Mn as their acetylacetonates	129
Twenty-three metal ions	S_7	M_{85}	Ascending technique ; run 10 cm; development time 20 min; activation at 100 ±5°C for three hours.	130
Polyphosphates	S_6	--	Detection of Polyphosphates in fish product.	131
Rare-earth elements and other metal ions	S_{49}	M_{86}	Ascending technique; 0.25 mm layer; run 170 mm; development time 100-110 min; correlation between R_F values and the paramagnetic moment of the rare-earths (III) is presented .	132
Ru, Au, Pd, Pt, Rh, Os, Ir, Ag	S_{55}	M_{87}	Qualitative analysis .	133
Fourteen rare-earth elements	S_5	M_{88}	The plates were first developed with solvent A, dried, and redeveloped with solvent B .	134
Heavy rare-earth metals	S_{56}	M_{89}	Separation of Cd, Tb, Dy, and Ho.	135
Rare-earth elements	S_{57}	M_{90}	Qualitative separations of rare-earth metals .	136
Pb, Fe^{+2},Ni, Co	--	M_{91}	Separation of metal ions from mixtures using four developing solvents .	137
Transition metals	S_{58}	--	Qualitative analysis .	138
Zn, Cd, Cu	S_5	M_{92}	Separations of Zn(II) from Cd(II) in presence of certain anions in the sample solutions of different pH .	139

Ion / metal	S.P.	M.P.	Comment	Ref.
$Fe^{2+}$and Fe^{3+}	S_7	M_{93}	Ascending technique; run 10 cm ; development time 30 min ; 0.25 mm layer ; sample pH 1.3 ; separation of Fe^{2+} from Fe^{3+} in presence of Cl^-,Br^-, SO_4^{2-} ,SO_3^- , PO_4^{3-} , and CH_3COO^- ions .	140
Polyphosphates	S_6	M_{94}	Densitometric determination of polyphosphates in meat products .	141
Fe, Cd, Zn, Cu, Bi, Tl^+, Ag, Hg, Pb, Se, Mn, UO_2^{2+}, Ni, Al, Zr, Ti, Th	S_7	M_{95}	Ascending technique ; run 10 cm ; development time 15-20 min ; 0.25 mm layer ; examination of effect of sample concentration , eluent concentration , pH of salt solution , pH of mobile phase and presence of anions in the sample solution on the separation of Cd^{2+} from Zn^{2+} and of Cu^{2+} from Ni^{2+}, Co^{2+} and Cd^{2+} with 1.0 M HCOONa - 1.0 M KI (9:1) .	142
Al, Fe^{2+}, Fe^{3+} Co , Ni, Cu, Zn, Ag, Cd, Hg, Tl. Pb, Bi	S_{59}	M_{96}	Ascending technique ; run 10 cm ; quantitative separation of Ni from Fe, Zn, Cd, and Pb .	143
Ni, Co, Zn, Cd, Cu, UO_2^{2+}, V^{4+}, Fe^{2+}, Fe^{3+}, Al, Th, Ti, Mo^{6+}, Sc, W, Hg, V^{5+}, Tl^+, Pb, Bi, Ag	S_7, S_{24}	M_{97}	Ascending technique ; run 10 cm; 0.25 mm. layer ; loading volume 5 mL ; examination of the effect of sample pH , presence of anions in the sample and the presence of alumina in the stationary phase on the separation of V^{5+} from W^{6+} and Mo^{6+} .	144
UO^{2+}, Ti, Al, W, Ni, Fe, Co, Mn, Cu, Bi, Zn, Cd, Ta, Se, Pb, Ce, Ag, Th, Zr, Hg, Tl	S_{60}	M_{98}	Ascending technique ; run 10-12cm ; development time 1-2 h ; qualitative separations .	145
Th, Zr, UO_2^{2+}	S_7	M_{99}	Ascending technique ; run 10 cm ; 0.25 mm layer ; studies on the effect of anions on the separations Th^{4+}-Zr^{4+}-	146

Ion / metal	S.P.	M.P.	Comment	Ref.
			UO_2^{2+} and Th^{4+}- Zr^{4+} .	
Light rare-earths	S_{28}	M_{100}	La, Ce, Pr, Nd, Sm, Eu, and Gd were completely separated ; detection limit 0.01 mg ; rare-earth R_F values decreased with increasing atomic number .	147
Rare-earth elements	S_{61}	M_{101}	R_F were governed by the amount of each component in the eluent ; discussion of the mechanism of separation REE ; application of the method to the separation of REEs in monazite.	148
Rare-earth elements	S_{61}	M_{102}	Qualitative separations ; detection limits 0.01 mg ; increase in R_F value with increasing volume of trimethyl ammoniun chloride in the mobile phase .	149
Rare-earths	S_5	M_{103}	Rare-earth R_F values in the range 0.1-0.7 ; separation of individual rare-earths from artificial mixture and from minerals and alloy samples .	150
Heavy rare-earths	S_{53}	M_{104}	La, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, and Y are separated from each other ; developer showed better separation efficiency .	151
Rare-earth elements	S_{62}	M_{105}	Separation of rare-earths by partition chromatography ; run 18 cm ; studies on the effect of pH of lactate solution, methanol concentration and temperature on the migration and resolution of the rare-earth elements .	152
La, Nd, Eu, Tb, Gd, By, Ho, Sm, Er, Tm, Yb, Pr	S_{63}	M_{106}	Separation of rare-earth elements in middle of atomic weight range .	153
VO^{2+}, Cr^{3+}, Mn^{3+}, Co^{3+},	S_5	M_{107}	Synthesis , spectroscopyand chromatography .	154
Twenty-eight metal ions in-	S_{64}	M_{108}	Quantitative separation of Ru^{3+} from other metal ions .	155

Ion / metal	S.P.	M.P.	Comment	Ref.
cluding Ru, Pd, W, Pt, Au, Mo, and UO_2^{2+}				
All rare-earths except Pm	S_5	M_{109}	Gives chromatograms showing typical separations of multicomponent mixtures containing adjacent lanthanides .	156
Rare-earth elements	S_{65}	M_{110}	Separation of lanthanides .	157
La, Ce, Pr, Nd, Sm, Gd, Dy, Er,Y, Pb	S_{53}	M_{111}	Qualitative separations and applications of the method to the analysis of rare-earth ores .	158
Noble metals (Rh, Ru, Pd, Pt, Ir, Au)	S_{66}	M_{112}	All noble metals were completely separated .	159
IO_3^-,IO_4^-, I^-, BrO_3^-, CNS^-, Oxalate ,AsO_4^{3-} PO_4^{3-}, $S_2O_3^{2-}$	S_7, S_{67}	M_{113}	Rapid microgram determination of ferricyanide (1-4 mg) and dichromate (2-10 mg) ions .	160
NO_2^-, I^-, IO_3^-, S^{2-}	S_6	M_{114}	Detection of anions at the nanogram levels under UV light .	161
La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu	S_{28}	M_{115}	Qualitative separations of rare-earths; plates were developed first with mobile phase I (ascent 8-14 cm ; dried at 40°C) and redeveloped in the same direction with mobile phase II to a distance of 20 mm .	162
Metal ions	S_{35}	M_{116}	Selective separation of Tl(III) from Cd, Al, Ni, Fe, Ag, and Pb .	163
Fe^{2+}, Fe^{3+}, Ni, Zn, Pb, Mn, Cu,	S_7	M_{117}	Reversed phase partition TLC of metal ions and examination of the effect of chlorosubstituent groups on R_F values .	164
Ni, Cu, Zn, Mn,	S_7	M_{118}	Studies on the effects of plate width , acetate concentration in mobile phase and time on the R_F values of the individual cations ; best separation with	165

Ion / metal	S.P.	M.P.	Comment	Ref.
			0.1 M sodium acetate , application of the method to the separation of metal ions in the tube-well water samples .	
Inorganic ions	S_{68}	M_{119}	Qualitative separations from multicomponent mixtures .	166
Forty-nine inorganic ions	S_{68}	M_{120}	The adsorption of most ions increases with increasing methanol content in the mobile phase .	167
Forty-nine inorganic ions	S_{68}	M_{121}	Selective separation of As(III) and Ti(IV) from many other ions .	168
Cu in Al-Fe-Ti alloy	S_5	M_{122}	Separation of Cu in Ferrotitaniun by concentration gradient TLC .	169
Al, Co, Ni, Zn, Ag, Cd, Tl, Pb, Bi, Mg, Hg, Fe, Cu	S_7, S_{69}	M_{123}	Much shorter development time on NH_4Cl - impregnated silica gel layers; clearer detection and more compact spot formation on KI-impregnated silica gel layers ; better results when KBr is used as eluant rather than as an impregnant , but reverse is true for KI , 1.0 M formic acid-1.0 M KBr (1+1) and formic acid-KI (1+9) were the best eluents for separating Fe^{2+} and Cu^{2+} from several metal ions , quantitative separation of Tl, Al Fe, Zn, and Cd .	170
Hg^{2+}, Hg_2^{2+}, Pb, Ni, Cu	S_{70}	M_{124} .	Recovery of Hg^{2+} from river water and industrial wastewater .	171
Twenty-seven cations	S_{71}	M_{125}	Qualitative separation ; effect of pH on the R_F values of metal ions in eluents containing lactic and citric acids .	172
Cu, Cd, Co, Pb	S_{72}	M_{126}	Quantification by densitometry ; detection limit in the nanogram range , the methods was applied to the determination of Cu and Pb in ground water and electroplating waste water .	173
All rare-earth elements including	S_4	M_{127}	Qualitative separations and quantification by densitometry , detection lim-	174

Ion / metal	S.P.	M.P.	Comment	Ref.
Y			its in the 14-31 ng. range .	
Alkali metals and alkyl xanthates	S_{73}	M_{128}	Method suitable for the analysis of floatation mixtures ; detection limit 0.5 mg .	175
Noble metals (Au, Pd, Pt, Ru, Ir, Rh)	S_{66}	M_{129}	Qualitative separations .	176
V, Mo, W, Re	S_{74}	M_{130}	Effective mutual separations of V^{5+}, W^{6+}, Mo^{6+}, and Re^{7+} with 0.5 M, HCl-ethanol (9+1) eluant ; optimum activation temperature 75°C for faster development ;starch as a binder causes tailing whereas dextrin , or solvar (polyvinyl alcohol) containing 10-14 % acetate groups gives better separations .	177
Ag, Ni, Cu, Co, Cd, Hg,	S_{75}, S_{76}	M_{131}	Separation of mixtures of inorganic ions on chitin layers ; possible use of chitin and chitosan layers in waste water and sea water purifying systems	178
Fe, Ni, Cu, Zn, Pb, Mn	S_7	M_{132}	Separations and detection of metal ions in industrial waste water samples	179
Co, Ni, Mn	S_{77}	M_{133}	Studies on the mobility of chlorides, carbonates and sulfates of the metals was investigated .	180
Fe, Ni, Cu, Pb, Zn, Mn	S_5	M_{134}	Examination of the effects of pH ; concentration of glycolic acid ; development time on the R_F values of individual cations in adsorption and partition TLC .	181
Au, Se, Tl	S_{24}	M_{135}	Simple and rapid separation of Au^{3+}, Se^{4+}, and Tl^{4+} .	182
Ni, Cu, Cd, Co, Zn	S_4	M_{136}	TLC separation and densitometric determination of Zn, Cu, Ni, and Cd at trace levels (0.01-0.3 mg) .	183
23 metal ions	S_{78}	M_{137}	Qualitative separations .	184

Ion / metal	S.P.	M.P.	Comment	Ref.
Lanthanides and other elements (Ce, Nd, Sm, Gd, Ho, Yb, Y, Ti, V, Zr, Th, U)	S_{79}	M_{138}	Examination of the effects of the concentration of H_2MEHP and HNO_3 on the R_F values of the elements .	185
Forty-nine inorganic ions	S_{68}	M_{139}	Separation of multicomponent mixture of ions .	186
Rare-earth metals	S_{80}	M_{140}	Qualitative separation by circular TLC.	187
Re, Mo, W, V	S_{24}	M_{141}	Separation of Re^{7+}, Mo^{6+}, W^{6+} and V^{5+} ions from their mixtures by TLC and electrochromatography ; investigation of the relationship between R_F values and concentration of acid in the mobile phase .	188
All rare-earths except Pm	S_5	M_{142}	Separation of multicomponent mixtures containing adjacent lanthanides, discussion of the effects of solvent cations and anions on R_F values .	189
Au, Pt, Pd, Cr, Mn, Fe, Co, Ni, Cu, Ba, Al, Bi, Pb, Zn, Ag	S_{17}	M_{143}	Application of method for the analysis of platinum powder and two kinds of Au alloy.	190
Metal ions of first transition series	S_{81}	M_{144}	Reversed phase TLC separations of metal ions , best separations at amine concentrations of 2 and 3 % in 0.03 M citric acid mobile phase .	191
Cu, Fe, Hg, Pd, Co	S_5, S_{82}	M_{145}	The mobility of metals generally increases on impregnated layers in the order phthalic, salicylic, syringic acid.	192
I^-,Br^-,IO_3^-,IO_4^- CH_3COO^-, NO_3^-, SCN^-,BrO_3^-, MoO_4^{2-},CrO_4^{2-}, $Cr_2O_7^{2-}$, $S_2O_3^{2-}$, $Fe(CN)_6^{3-}$, $Fe(CN)_6^{4-}$	S_7	M_{146}	Acetone , ethyl methyl ketone , acetophenone , butan-2-ol, and formic acid-ketone systems were most suitable for providing compact and clear separations .	193

Ion / metal	S.P.	M.P.	Comment	Ref.
Inorganic phosphates	S_6	M_{147}	Qualitative separations .	194
Br^-, I^-, VO_3^-, NO_3^-, NO_2^-, SCN^-, CrO_4^{2-}, $Cr_2O_7^{2-}$, WO_4^{2-}, MoO_4^{2-}, PO_4^{3-}, $Fe(CN)_6^{3-}$, $Fe(CN)_6^{4-}$	S_7, S_{83}	M_{148}	Aqueous sodium chloride-acetone , aqueous ammonia- acetone (9+1) were the most effective solvent systems for differential migration of anions .	195
Forty-nine inorganic ions	S_{84}	M_{149}	Qualitative separations .	196
Inorganic ions	S_{85}	M_{150}	Selective separation of Re (VII) from many inorganic ions .	197
Zr, Hf	S_5	M_{151}	Complete separations of the two metals present in Zr:Hf ratios ranging from 20:1 to 1:40 .	198
Several metal ions	S_6, S_{86}	M_{152}	Separation of metal ions with different valence states .	199
Fe, Ni, Zn, Cu, Pb, Mn	S_7	M_{153}	Studies on the dependence of R_F values on the migration time ; pH and concentration of the mobile phase ; quantitative determination of separated metal ions by atomic absorption spectroscopy .	200
Forty-nine inorganic ions	S_{84}	M_{154}	Qualitative TLC	201
Ce^{2+}, Ce^{4+}, Nd, Eu, Gd, Tb, Yb, Y, Ti, V, Zr, Th	S_{87}	M_{155}	The R_F values of the lanthanide ions increase with increasing concentrations of citric acid in the mobile phase.	202
Alkali metals	S_{88}	M_{156}	Qualitative separations .	203
Rare-earth elements	S_{89}	M_{157}	Qualitative separations .	204
Cu, Co, Cd, Hg, Ni, Ag	S_{75}, S_{76}	M_{158}	Qualitative separations and determination of chromatographic parameters as a function of the concentrations of MeOH, NH_3, AcOH, and inorganic salts in the mobile phase .	205

Ion / metal	S.P.	M.P.	Comment	Ref.
Ni, Cu, Zn , Pd , Cd ,Cr, Fe ,Ru, Rh, La ,Au ,Tl, Zr, Pt,Nb, Ta,Mn, Ag ,Hg, Co ,Mo, W	S_7, S_{90}	M_{159}	Run 11 cm ; correlation between R_F values on impregnated layers developed with DMSO- THF (1+10) and the atomic numbers of the metal ions.	206
Transition metal ions	S_{75}, S_{76}	M_{160}	Qualitative TLC	207
Twenty One inorganic cations	S_6	M_{161}	Separation and identification of cations on cellulose layers using six detection reagents .	208
IO_3^-, IO_4^-, BrO_3^-, I^-,MoO_4^{2-}, $Fe(CN)_6^{4-}$	S_5	M_{162}	Effect of heavy metals on the chromatographic separation of periodate from iodate ,bromate ,iodide , molybdate and ferrocyanide.	209
Eighteen anions	S_{24}, S_{91}	M_{163}	Investigations of the effect of transition metals on the separations Cl^-- Br^- - I^- and NO_2^- -NO_3^- .	210
Seventeen anions	S_{92}	M_{113}	Qualitative separations .	211
I^-,Br^-, NO_2^-,IO_3^-, BrO_3^-, NO_3^-, VO_3^- MnO_4^{2-}, SCN^-, CrO_4^{2-}, $Cr_2O_7^{2-}$, WO_4^{2-}, MoO_4^{2-}, oxalate, PO_4^{3-}, $Fe(CN)_6^{3-}$	S_{93}	M_{164}	Microgram detection and separation of anions .	212
Fe, Cu, Mn	S_6	M_{165}	Qualitative separations .	213
Pb,Cd, Zn	S_{94}	--	Application of TLC - square -wave anodic stripping voltametry for the determination of heavy metals .	214
Mn, Co, Ni, Cu, Zn, Fe , Cr, Ti, V	S_{81}	M_{166}	Reversed -phase TLC for qualitative identification of 3d metal ions .	215
Eleven metal ions	S_{95}	--	Retention behavior of metal ions from aqueous solutions at pH 1-7 on impregnated silica layers .	216

Ion / metal	S.P.	M.P.	Comment	Ref.
Metal ions	S_{97}	M_{167}	Qualitative separations .	217
Mg, Al ,Ca,V, Cu, Zn, Ge, Y,Zr, Mo, Ag, Cd, In, La, Ce, Eu, Tb, Tl, Pb, Bi	S_{98}	M_{168}	Detection limits and R_F values of fluorescent cations separated on porous glass sheet .	218
Forty- nine inorganic ions	S_{99}	M_{169}	Separation of Sc(III) , rare -earths (III), Y (III) , Th(IV) and V(VI) from other ions .	219
Fe,Co,Zn, Cd,Cu, Ni,	S_{96}	--	Application to the analysis of alloys and natural water samples .	220
Cd	S_{100}	M_{162}	Study of the influence of soil properties and constituents on the mobility of cadmium by soil TLC .	221
Rare elements	S_{80}	M_{170}	Preconcentration of rare- earths by circular TLC for subsequent ICP-AES determination in geological samples.	222
Toxic metal ions	S_5, S_{60}, S_{101}	M_{171}	Normal and reversed phase and chelation TLC of some toxic metal ions ; quantitative separation of Pb from binary mixtures and synthetic alloys .	223
Twenty- six transition and alkali metal ions	S_6	M_{174}	Qualitative separations .	224
Heavy metals	S_{106}	--	Quantitative separation of Fe (III) , Cu(II) and Pb(II) from other metals .	225
Some anions	--	--	Use of acid phosphates for detection and determination of typical anions .	226
Some anions	S_7, S_{24}, S_9, S_{91}	M_{162}	Qualitative separations ; effect of $CaCl_2$, $MgCl_2$ and $NaHCO_3$ on the separation of anions ; identification of NO_2^- in artificial sea water .	227
Cl^-, Br^-,I^-,ClO_3^-, ClO_4^-, $H_2PO_4^-$, NO_2^-, NO_3^-, SCN^-, SO_4^{2-}	S_{107}	M_{178}	Separation of inorganic anions as DMA (diantipyrilmethane) complexes and salts of protonated DMA using radial or ascending technique ; determination of anion content by	228

Ion / metal	S.P.	M.P.	Comment	Ref.
			planimetry using calibration plots of zone area against amount of anion .	
Forty-nine inorganic ions	S_{102}	M_{172}	R_F value increases with increasing acid or sulfate in the mobile phase .	229
3d series transition metal ions	S_{103}	M_{173}	Examination of the effect of the concentration of H_2MEHP and HNO_3 on R_F values .	230
Uranium	S_{104}	M_{175}	Ascending technique ; selective separation of uranium from synthetic mixture of several metal ions .	231
Cations with some anions	S_{79}	M_{176}	TLC of cations and anions , qualitative separations .	232
Inorganic ions	S_{105}	M_{177}	Separation of polyvalent ions and trivalent rare earths which tend to form anionic sulfate complexes .	233
Inorganic metal ions	S_{108}	M_{179}	The effect of impregnants and of mobile phases of different pH on the mobility of inorganic ions .	234
Copper	S_7	M_{180}	Layer thickness 0.25 mm ; plate activation at 110° C from biological tissue by dryoxidation and ashing , then chromatography on silica .	235
Hg(II),Cu(II) , Cd(II)	S_{47}	M_{181}	Temperature 25°C , UV spectroscopy determination of metal ions after elution with H_2O .	236
Au(III) , Ru(III), Rh(III), Pd(II), Os(IV) , Pt(IV)	S_5	M_{182}	R_S value of each pair of ions is 1.0 except that of Ru(III) and Pd(II) , limit of detection is 4.0 μg .	237
Inorganic ions	S5	M_{183}	Relationship between volumetric % concentration (Cp) of IBMK / FA and the R_F values of ions is obtained.	238
Inorganic ions	S_9 , S_{109}	--	Reactions of inorganic ions with organic reagents are studied on thin layers .	239
Rare- earths	S_{110}	M_{184}	R_F value of each metal decreased with increasing pKa value of amine used for pretreatment .	240

Ion / metal	S.P.	M.P.	Comment	Ref.
Actinides	S_{111}	--	Actinides are separated on the basis of different sorption behavior in (III) and (V) oxidation states .	241
Th^{4+}, Fe^{3+}, Al^{3+}, Bi^{3+}, UO_2^{2+}, Cd^{2+}, Hg^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Pb^{2+}, Ag^{+}, Tl^{+}	S_{112}	M_{185}	Ascending technique ; run 10 cm ; layer thickness 0.25 mm ; limits of detection falls in the range of 0.22 - 3.4 µg .	242
Cd^{2+}, Zn^{2+}, Cu^{2+}, Pb^{2+}	S_5	M_{186}	Ascending technique ; run 10 cm. ; quantitation of Cu(II) by AAS after separation from other metal ions .	243
Transition metal ions	S_{113}	M_{187}	Separation of eight component mixture ; quantitative estimation by AAS.	244
I^-, IO_3^-, IO_4^-, Br^-, BrO_3^-, NO_2^-, SCN^-, CrO_4^{2-}, PO_4^{3-}, MnO_4^-, WO_4^{2-}	S_{114}	M_{188}	Ascending technique ; semiquantitation of I^-, Br^- and NO_2^- by spot area measurement .	245
I^-, IO_3^-, IO_4^-, Br^-, BrO_3^-, NO_2^-, MnO_4^-, CrO_4^{2-}	S_5, S_9, S_{21}, S_{115}	M_{189}	Ascending technique ; run 10 cm. ; semiquantitation of IO_4^- by peak height measurement .	246
Thirty cations	S_{116}	M_{190}	Development time 19min. ; separation of Pt from 27 other cations .	247
Cd(II), Cu(II), Pb (II)	--	--	Detection limits for Cd (II) and Pb is 1 and 4 µg respectively ; on plate square wave stripping voltametry .	248
Heavy metal	--	--	Separation and identification of metals in human bones ; placenta ; milk and air by adsorption and IE-TLC .	249
Ni, Co, Cu	--	--	Determination in rock samples by TLC / photodensitometry ; relative error ranging from 2 % to 38% .	250
Co(II), Fe(II), Cu(II)	S_{117}	M_{191}	Some new TLC plates are suggested to immobilize various ion - attractive meterials .	251

Ion / metal	S.P.	M.P.	Comment	Ref.
Al, Ca, Co, Cr, Cu, Fe ,Mg, Mn, Mo, Ni,Pb, Sn, Ta, Ti, V,Y,Zr	S_{118}	--	ICP-AES determination in Zr-U alloys after separation by TLC .	252
Metal cations	--	--	Determination of Fe in process media by employing 8-hydroxyquinoline as complexing agent ; Lumogallion was used in Al detection.	253
SCN^-, MnO_4^-, NO^{2-}, CrO_4^{2-}, CrO_7^{2-},MoO_4^{2-}, $Mo_7O_{24}^{6-}$, $Fe(CN)_6^{4-}$, $Fe(CN)_6^{3-}$, Fe^{3+}, Zn^{2+},Cu^{2+}, Ca^{2+}, Co^{2+}, Hg^{2+}, UO_2^{2+},VO^{2+}, Ag^+,Mn^{2+}, Bi^{3+}	S_{119}	M_{192}	TLC separation and colorimetric determination of SCN^- as applied to water and wastewater .	254

S.P. : Stationary Phase

M.P. : Mobile Phase

Table 2.2 : List of stationary phases used

S_1 Silanized silica gel (Merck , Darmstadt) and polygram plate

S_2 Precoated plates; Fixion 50× 8 (H^+ or Na^+) saturated with mobile phase vapours

S_3 Cellulose phosphate (Whatman P 41 , U.K.) and cellulose phos phate + microcrystalline cellulose (3:1)

S_4 Silica gel H

S_5 Silica gel

S_6 Cellulose

S_7 Silica gel G

S_8 Silica gel impregnated with diethylenetriamine

S_9 Cellulose microcrystalline

S_{10} Zirconiun tungstate

S_{11} Cellulose MN 300 G Containing 10% $CaSO_4$

S_{12} Lanthanum antimonate (binder-free)

S_{13} Hydrated stannic oxide

S_{14} Silufol 254

S_{15} Silica gel with starch binder and fluorescent indicator

S_{16} DEAE Cellulose

S_{17} Ecteola-cellulose

S_{18} Silica gel and vionit CS- 32 ion-exchange resin

S_{19} Dowex 1 or 50 mixed with cellulose (Avicel SF)

S_{20} Dowex 50 × 8 cation - exchange resin

S_{21} Alumina G

S_{22} Sulfoethyl cellulose (Serva, Heidelberge , FRG)

S_{23} Ti (IV) antimonate (H^+ form)

S_{24} Alumina

S_{25} Glass powder

S_{26} Corn starch

S_{27} Cellulose MN 300

S_{28} Mixture of silica gel , starch and NH_4NO_3

S_{29} Silica gel with hydrous MnO_2

S_{30} Stannic arsenate

S_{31} Silica gel impregnated with 0.5% sulfaguanidine

S_{32} Silica gel impregnated with 8-hydroxyquinoline and dibenzoylmethane

S_{33} Sulfoethyl cellulose (strongly acidic) cation exchanger

S_{34} Silica gel R and 15 % vionit CS ion exchanger

S_{35} Silica gel impregnated with Aq. sodium molybdate

S_{36} Silica gel impregnated with organophosphorus compounds

S_{37} Cellulose phosphate in H^+ form (Whatman P 41, U.K.)

S_{38} Silica gel impregnated with 0.5 % aqueous 2, 2-dipyridyl and iminodiacetic acid

S_{39} Hydrous zirconium oxide

S_{40} Silica gel impregnated with 30% *s*-or *t*-butylamine

S_{41} Silica gel impregnated with crystalline antimonic (V) acid-*p*-sulfochlorophosphonazo

S_{42} Tin (IV) arsenosilicate and arsenophosphate

S_{43} Semicrystalline Sn phosphate ion - exchanger + silica gel G

S_{44} Silica gel impregnated with fluorescein

S_{45} Zirconium (IV) molybdate

S_{46} Polyamide

S_{47} Silica gel 60

S_{48} Silica gel (Wakogel B- 0)

S_{49} PEI cellulose

S_{50} Silica gel G mixed with chitosan

S_{51} Cellulose with azopyrocatechol group

S_{52} Silica gel impregnated with 0.5 M NH_4Cl and a saturated Aq. solu tion of barium nitrate

S_{53} Silica gel- ammonium nitrate - CM cellulose (5.0 : 0.64 : 0.16 w/w)

S_{54} Silica gel H- ammonium nitrate - CM cellulose (5.0 : 0.64 : 0.16 w/w)

S_{55} Tin pyrophosphate and silica gel containing sodium CM cellulose as binder

S_{56} Silica gel- sodium CM cellulose - ammonium nitrate

S_{57} Silica gel - ammonium nitrate - sodium CM cellulose -water , (33 : 4 : 1 : 100 w/w)

S_{58} Silica gel impregnated with hydroxybenzoic acid

S_{59} Silica gel impregnated with 0.1 M Aq. sodium nitrite , sodium molybdate and potassium dihydrogen orthophosphate

S_{60} Silica gel loaded with various concentrations of TBA

S_{61} Silica gel - starch - ammonium rhodanate (2.8 : 0.15 :0.5 w/w)

S_{62} C_{18}- bonded silica reversed phase layer

S_{63} C_{18}- bonded silica

S_{64} Binder free Zr (IV) antimonate in the H^+ form , silica gel G and mixture of Zr (IV) antimonated and silica gel G (1:1)

S_{65} Silica gel H - microcrystalline cellulose - ammonium nitrate

S_{66} Microcrystalline cellulose modified with silica gel G

S_{67} Antimonic acid

S_{68} Cellulose (MN 300) impregnated with PEI

S_{69} Silica gel impregnated with 0.1 - 1.0 M Aq. solutions of NaCl , NH_4Cl , KBr or KI

S_{70} Silica gel treated with acid

S_{71} Stannic silicate

S_{72} Silica gel H and sodium CM cellulose

S_{73} Silufol UV^{254} impregnated with 5 % paraffin oil in hexane

S_{74} Polychrom (poros polymer , particle size 10- 40 μ) in combination with polyvinyl alcohol

S_{75} Chitin

S_{76} Chitosan

S_{77} Silufol sheets (aluminium- backed silica gel)

S_{78} Zirconium phosphoantimonate

S_{79} Silica gel impregnated with different concentrations of mono - 2 - ethylhexyl acid phosphate (H_2MEHP)

S_{80} Fixion 50 × 8

S_{81} Silica gel coated with high molecular weight amines (Primine JM-T, Amberlite LA - 1, Alumina 36, Aliquat 336)

S_{82} Silica gel impregnated with salicylic , syringic or o- phthalic acids

S_{83} Silica gel impregnated with copper sulfate solution

S_{84} p - Amino benzyl cellulose

S_{85} Diethyl - (2 - hydroxypropyl) aminoethyl Q E - cellulose (strong basic anion exchanger)

S_{86} Synthesized carbamide - formaldehyde polymer (amino plast)

S_{87} Silica gel coated with different concentraton of Primine JM - T

S_{88} Zinc ferrocyanide

S_{89} Diatomite

S_{90} Silica gel impregnated with DMSO

S_{91} Alumina - silica gel G (1 : 1 , 1 : 2 , 2 : 1)

S_{92} Anhydrous antimony (V) oxide

S_{93} Silica gel impregnated with 0.1 % Aq. solution of copper sulfate, zinc sulfate , nickel chloride , cobalt chloride , Co $(NH_3)_6Cl$

S_{94} CM cellulose

S_{95} Silica gel impregnated with a mixture of alizarin red S and aliquat 336

S_{96} Silica gel modified with analog of dibenzo- 18 - crown - 6

S_{97} Mixtures of silica and inorganic ion-exchange gels

S_{98} Poros glass sheets

S_{99} Diethyl - (2 - hydroxypropyl) aminoethyl cellulose

S_{100} Twentytwo soils with different characteristics

S_{101} Silica gel impregnated with TBP

S_{102} Arsenosilicates of Sn (IV) , Cr (III) and Sb (V)

S_{103} Silufol

S_{104} Silica gel impregnated with high molecular weight amines

S_{105} Silica gel impregnated with mono-2-ethylhexyl acid phosphate (H^+ - MEHP)

S_{106} Stannic sulfosalicylate

S_{107} Trimethyl hydroxypropylamine cellulose (QA)

S_{108} Surface - modified sorbent layers

S_{109} Silica gel GF_{245}

S_{110} Silica gel pretreated with amines {(iminodi-2 ethanol ; tris (hydroxymethyl) aminomethane ; nitrilotri-2-ethanol and 1, 3-diazole}

S_{111} silica gel G impregnated with polyethylene glycol

S_{112} Silica gel and alumina impregnated with 0.1-1.07 LiCl

S_{113} Silica gel plain and impregnated with EDTA (2%), DMG(1%) or1,10-phenanthroline (1%)

S_{114} Silica gel plain and impreganted with 1% Aq. $CuSO_4$ / Alumina , Kieselguhr , Kaolin , Alumina + cellulose (2 :1) Kieselguhr + cellu lose (1:2,2:1)

S_{115} Kieselguhr / Kieselguhr+ Cellulose (4:1,3:2)

S_{116} Ce(III) silicate

S_{117} Silica gel impregnated with sodium salt of condroitin sulfate

S_{118} TBP coated polymeric supports

S_{119} Cellulose microcrystalline / cellulose + Kieselguhr (4:1 , 3:2 , 1:1) ; Kieselguhr

Table 2.3 : List of Mobile Phases Used

M_1 Et_2O -bis (2- ethylhexyl) phosphate -HNO_3(100+3+2);diiso - Pr_2O - THF - HNO_3 (25+20+1) ; Et_2O - bis - (2- ethylhexyl) phosphate - HNO_3 (50+2+1)

M_2 1.0 -2.0 M Sodium nitrate

M_3 Aq. acetic acid (0.1-3.0 M) and mixtures of acetic acid and ammonium acetate solutions

M_4 N- butylacetate - conc. HCl (100+1.5)

M_5 Polar and nonpolar solvents

M_6 Me_2SO - HCl (1-6 M) (1+9 , 3+7, 5+5,7+3and 9+1, v/v)

M_7 Et_2O - bis (2- ethylhexyl) phosphate - HNO_3(100+1+3.5), Et_2O - THF - bis (2- ethylhexyl) phosphate - KNO_3 (10+15+1+3.5)

M_8 Ten Aq. and mixed solvent systems

M_9 Mixture of short - chain alcohols and /or acetone with strong or weak acids

M_{10} Seventeen solvent mixtures

M_{11} Dioxane

M_{12} Buffered Aq. ammonia (pH 10)

M_{13} Concentrated Aq. ammonia - n- propanol (1+2), pyridine - water - n - butanol (3+3+5)

M_{14} MeOH - benzene - ethylacetate - EMK- Aq. ammonia (6+6+ 6+2+1)

M_{15} Acetone - benzene (1+1)

M_{16} 5M HCl with different concentrations of hydrogen peroxide

M_{17} Mixtures of Me_2CO ,HCl and water in various ratios

M_{18} Different concentrations of HCl and Aq. chloride solutions of Li, Mg, Na , Ca, Sr and Ac

M_{19} Et_2CO - bis (2 - ethylhexyl)phosphate - HNO_3 -Et_2 CO - THF , THF - bis (2 - ethylhexyl) phosphate - HNO_3 and iso - Pr_2 CO - THF-HNO_3

M_{20} HOAc - HCl

M_{21} Me_2CO - EtOAc - C_6H_6 , (7+1+3)

M_{22} Iso - Pr_2O - THF - conc. HNO_3 - H_2O (100+70+4.21+3.45)

M_{23} Aq. HCl

M_{24} Polar solvent systems

M_{25} Water , Aq. solutions of NH_3 , KCl , NaF , KNO_3 , K_2SO_4 ,$KClO_4$-Na_2CO_3 and K_3PO_4 at different molar concentrations

M_{26} Distilled water , Aq. sodium chloride (0.1-1%)

M_{27} Butanol - acetone - water (9+9+2)

M_{28} Acetone - water (10+1), methanol - water - butanol (3+1+1) ,butanol saturated with 2N HNO_3

M_{29} 1.0 M acetic acid and mixtures of 0.1 M acetic acid and ammonium acetate (pH 4.7,5.7, 6.5 and 6.7)

M_{30} Butanol - propanol - water (1+3+1)

M_{31} Many single - component and mixed solvent systems containing polyhydric alcohols , methylamine , Aq. ammonia , DMF ,water , Ketones , pyridine and esters

M_{32} Acetone - butanol - 10% Aq. ammonia - water (13+4+2+1)

M_{33} HNO_3 (0.001-1.0 M) , DMF - 0.1M HNO_3 (1+0, 4+1,1+4, 4+6, 6+4)

M_{34} 0.1 M ammonium oxalate , DMSO - 1M HCl (1+9)

M_{35} Binary solvent systems

M_{36} 3 or 5 M HCl containing $NaClO_4$

M_{37} Different solvent mixtures

M_{38} Aq. and Aq. - organic binary mixtures in various ratios

M_{39} TBP -EMK , EtOAc and HNO_3

M_{40} Dil. HNO_3

M_{41} Aq. HCl

M_{42} Isopropanol - ethylacetate - DMF - acetic acid - water (10+ 6+1+1+2), iso - propanol - ethylacetate - DMF - acetic acid - water (60+30+3+5+10)

M_{43} Propanol - Aq. ammonia (2+1)

M_{44} THF - acetic acid -water (10+1+1)

M_{45} Acidic solvent systems

M_{46} Butanol - ethyl acetate - acetic acid (4+1+1) and ethyl acetate - formic acid - water - pyridine (3+1+1+0.5)

M_{47} 0.05 M H_2SO_4 - methanol or acetone (1+0,4+1,3+2 or 2+3 v/v)

M_{48} Oxalic acid - Oxalate systems

M_{49} Mixtures containing ether ,THF , bis (2- ethyl hexyl) phosphate , and HNO_3 in different ratios

M_{50} Me_2CO - HCl - water (45+2+3) and BuOH - HCl - water (45+4+6)

M_{51} Monotetradecyl phosphate (P 538) , ethylacetate and HNO_3

M_{52} 6M HCl - acetylacetone - 9M H_2SO_4 (60+2+1)

M_{53} Demineralized water , mixtures of sodium formate and formic acid solutions in different ratios

M_{54} Acetone - n - butanol - conc. Aq.ammonia - water (3+4+2+1) and n- butanol - ethanol - conc. Aq. ammonia - water (2+2+1+1)

M_{55} 20% acetic acid - dioxane - 88% isopropanol - 9% trichloroacetic acid - methanol (1+2+3+5+10)

M_{56} MeOH - 6N HNO_3 - acetylacetone (4+3+1)

M_{57} 0.5M H_2SO_4 - acetone or methanol (1+0, 4+1, 3+2 and 2+3)

M_{58} Isoamyl alcohol - water - AcOH (2+1+1) ,methanol - benzene - AcOH (4+2+3)

M_{59} Aq. solutions of inorganic acids ($HClO_4$, HCl , HBr, HNO_3 , H_2SO_4 and H_3PO_4) , their mixtures and some of their salts

M_{60} Aq. HCl and HNO_3 of different pH and their mixtures with organic solvents in different ratios

M_{61} 1.0M HCOOH , 1.0M HCOONa and their binary mixture (1+1,2+8, 4+6, 6+4 , 8+2)

M_{62} Aliphatic alcohol - HCl systems

M_{63} Isopropyl ether - THF - tributyl phosphate - HNO_3 (10+6+1+1)

M_{64} Iso - PrOH - Et_2O - EtOAc - HNO_3 (20+20+10+1) ,iso - PrOH-THF-HNO_3 (30+10+1)

M_{65} Potassium pyrophosphate solution

M_{66} Buffered EDTA solution

M_{67} Pyridine - water -n- butanol - Aq. ammonia (4+8+2+1)

M_{68} Toluene - acetic acid (5+2)

M_{69} Butanol - formic acid (1+1)

M_{70} Chloroform or Toluene

M_{71} Mixture of organic solvents

M_{72} 20.0 M formic acid in butanol

M_{73} Ammonium nitrate solutions of different concentrations (0.5 - 6.0M) with constant pH (4.9+0.1)

M_{74} Ethanol - HCl (4+1, 9+1) ; ethanol - HNO_3 (9+1) ; isopropanol - HCl (9+1) and ternary mixtures of organic ,Aq. and mineral acids in different ratios

M_{75} 0.01 - 1.0M HCl

M_{76} n- butanol - HCl (2+1,3+1, 4+1, 9+1, 17+3)

M_{77} Aq. solutions of HCl (0.1 - 0.5 M) buffer solutions of different pH ,Aq. - organic mixtures of different composition

M_{78} Aq. solutions HCOOH, HCOONa , CH_3COOH ,CH_3COONa , CH_3COOH- CH_3COONa (1+1) and HCOOH - HCOONa (1+1)

M_{79} Aq. formic acid solutions containing CH_3COOH , DMSO or EMK ; IBMK saturated with formic acid

M_{80} Aq. H_2SO_4 (0.01 - 0.1 M) and H_2SO_4 - $(NH_4)_2 SO_4$ (0.01 - 1.0 M) solutions

M_{81} Phosphate - Et_2O - HNO_3 (40+40+0.8 ; 20+42+2.5)

M_{82} P_{204}- dioxane - EtOAc - HNO_3 (1+1+2 ; 0+2.4+4.0)

M_{83} TBP - THF - Et_2O - HNO_3 (1+9+9+1.5)

M_{84} Hydrocarbons and their mixtures in different ratios

M_{85} 0.05 - 2.0 M formic acid in butanol

M_{86} Mixtures of 0.05 M HCl and 0.01 - 1.0 M NH_4SCN in different ratios

M_{87} Acids(nitric , tartaric , citric , perchloric , formic) , bases {NH_4OH , $(CH_3)_3N$} , neutral compounds (NH_4Cl , NH_4NO_3, $AcONH_4$) or mix ture of these with organic solvents (EtOH, MeOH, n- PrOH , Me_2CO)

M_{88} Monotetradecylphosphoric acid - Et_2O - conc. HNO_3 (20+42+2.5 ; 40+40+0.8)

M_{89} Et_2O - THF - bis (2- ethylhexyl) phosphate - HNO_3

M_{90} P_{507} - IBMK - iso - (Pr_2O - HNO_3 (1+12+6+2.4 v/v)

M_{91} n - propyl alcohol - ethyl acetate - conc. HNO_3 (10+30+1) , n-propylalcohol - dichloromethane - conc. HNO_3 (10+30+1 , 30+10+1), n- propylalcohol - cyclohexane anhydrous alcohol- conc. HNO_3 (20+15+15+1),n- propyl alcohol - petroleum ether - THF - conc. HNO_3 (25+15+15+1)

M_{92} Mixed organic solvents containing s-butylamine

M_{93} 10M HCOOH in butanol - ethylemethyl ketone (1+1,2+1, 1+3,3+1)

M_{94} Isopropanol - 13.5 % trichloroacetic acid - 25% Aq. ammonia (140+40 +0.6)

M_{95} Aq. HCOONa (10^{-3}, 10^{-2},0.1, 1.0 and 5.0M) , 0.1M HCOONa- 0.1M HCOOH (1+9 ,3+7,1+1,7+3 and 9+1) , 1.0M HCOONa - 1.0M NaCl (1+1,1+2,2+1,1+9and 9+1) mixture of 0.1 or 1.0M HCOONa and 0.1 or 1.0M NaCl , KBr , KI or $KBrO_3$ in different ratios

M_{96} Mixtures of Aq. 1.0M formic acid and alkaline salt solutions

M_{97} Ternary mixtures of different ratios of TBA , formic acid , and acetate

M_{98} Aq. formic acid (10^{-3} - 2.0M) , Aq. sodium formate (10^{-3}-5M) and mixtures of 1.0M formic acid and 1.0M sodium formate (1+1,4+6,6+4,2+8 and 8+2)

M_{99} Binary mixtures containing DMA and distilled water ,methanol ; or butanol ; ternary mixtures containing DMA, acetone or ethyl acetate and formic acid

M_{100} Trialkylmethyl ammoniumchloride -n- octyl alcohol - petroleum ether - conc. HNO_3 (60+7+25+1)

M_{101} Tri -n- octylamine or tri- iso- octylamine -4- methyl -2- pentanone - isopropyl ether - isopropyl alcohol - NHO_3 (1+8+8+6+0.75,v/v)

M_{102} Tri methyl ammonium chloride -n- octyl alcohol - petroleum ether- HCl (2+10+30+1, 2+6+30+1.2 and 2+7+30+1 ,v/v)

M_{103} P_{204} - P_{507} - tributyl phosphate - THF - HNO_3 - diisopropyl ether (111+52+5+521+86+1000 and 68+43+27+460+103 +1000 ,v/v)

M_{104} I. Bis (2 - ethylhexyl) phosphate - diisopropyl ether - diethyl ether - nitric acid (1+10+6+1.1)

II. Mono (2- ethylhexyl) phosphate - isopropyl ether - ethyl ether - nitric acid (1+8+8+1.1)

M_{105} Methanol - lactate media

M_{106} Mixed Aq. - hydroxyisobutyric acid solutions containing methanol

M_{107} Methanol - CH_2Cl_2 (1+99)

M_{108} HNO_3 (10^{24} - 1.0M) DMSO - 0.1M HNO_3 (1+0, 4+1, 3+2, 1+4), dioxane - 0.1M HNO_3 (1+0, 4+1,3+2,2+3,1+4)

M_{109} Aq. alkali metal nitrate solutions

M_{110} Bis- (2- ethylhexyl) phosphate [P_{204} + 2 - ethylhexyl (2- ethyl) phos phoric acid (P_{507}) - THF - HNO_3 - isopropyl ether ,3+2+90+19+280, 17+2+110+18+20 , v/v]

M_{111} Mixed solvent systems consisting of mono (2- ethylhexyl phosphate, 4- methyl pentanone , nitric acid , isopropyl ether and /or THF in dif ferent ratios

M_{112} n- Butanol - HCl - acetone (100+1+100)

M_{113} Aq. organic acids

M_{114} Aq. sodium salicylate

M_{115} (I) P_{507} - mono (2- ethylhexyl) phosphonate- diethyl ether - conc. HNO_3 (1.5+43.5+2) and

(II) P_{507} - diethyl ether - conc. HNO_3 (3+42+1)

M_{116} Formic acid - butanol systems

M_{117} Aq. solutions (0.01 -0.2M) of acetic acid and chlorosubstituted (mono - , di- and trichloro) acetic acids

M_{118} Aq. solutions of sodium acetate

M_{119} Mixed HCl - organic solvent systems (0.1M HCl - ethanol or acetone (1+0, 7+3, 1+1, 3+7)

M_{120} Mixed H_2SO_4 - organic (methanol or acetone) solvent systems

M_{121} 0.1M HCl - dioxane (7+7, 5+5, 3+7 v/v)

M_{122} Isopropanol - THF - 50 % HNO_3 (60+20+3)

M_{123} Demineralized water , 0.1M formic acid ; 1.0M KI , KBr or NaCl ; 0.1M formic acid - 0.1M KI or KBr (1+9) , 1.0M formic acid - 1.0M HBr (1+9 and 9+1) ,1.0M formic acid - 1.0M NaCl , NH_4Cl ,KBr or KI (1+9,3+7,1+1,7+3 and 9+1)

M_{124} Ethyl acetate - acetone , formic acid - water (4+1 ,8+7)

M_{125} Thirty six solvent systems

M_{126} 0.2M acetic acid - 0.2M sodium acetate systems

M_{127} Organic solvent mixtures consisting of di - (2 - ethylhexyl) phosphate, mono - (2- ethylhexyl) phosphonate , tributyl phosphate , THF ,nitric acid and isopropylether in different ratios

M_{128} 10% ammonium acetate with Aq. ammonia

M_{129} n- BuOH - IBMK - p^{20} - HCl (140+100+15+18) and (140+100+15+1)

M_{130} Mixtures of EtOH andAq. NaCl or HCl

M_{131} Aq. methanolic solutions (1+1.3+1 ,v/v) of ammonia or AcOH with ammonium nitrate , or sometimes ammonium acetate

M_{132} Aq. solutions of sodiummalonate and sodium malate

M_{133} Distilled water and buffer solutions (pH 5.2, 7.2 or 9.2)

M_{134} 0.05M glycolic acid at pH 2.5 (adjusted with HCl)

M_{135} Aq. solutions of HCl , HBr , H_2SO_4 , HNO_3 , H_3PO_4 and various organic acids

M_{136} MeOH - EtOH - NH_4Cl - Aq. ammonia

M_{137} Buffered EDTA solutions

M_{138} HNO_3 (0.05 - 3.0M)

M_{139} Mixed H_2SO_4 and dioxane systems

M_{140} Oxalic acid and NH_4Cl solutions

M_{141} Aq. solutions of organic and inorganic acids

M_{142} Aq. solutions of alkali - metal chlorides

M_{143} Mixtures of 2.5M HCl , 2.5M NaCl and 0.6 % hydrogen peroxide in different ratios

M_{144} 0.03M citric acid

M_{145} Distilled and tap water

M_{146} Sixty - nine organic solvent systems (single component , two component ,and three component systems comprising amine, alcohols, ketones or phenols and formic acid)

M_{147} Water - ethanol - 2- methyl propanol - 2 - propanol - Aq. ammo

nia - trichloro acetic acid (150+175+75+107+2+25)

M_{148} Acetone mixed with 0.1M Aq. HCl ,NaCl , HBr , formic acid orAq. ammonia (1+9 or 9+1)

M_{149} H_2SO_4 (0.01 - 1.0M) and H_2SO_4 - ammonium sulfate (0.01 - 1.0M) systems

M_{150} Aq. HCl and HCl - ammonium chloride (0.01 - 1.0M) systems

M_{151} HNO_3 - HCl or H_2SO_4 containing different concentrations of hydro gen peroxide

M_{152} Eleven neutral and acidic solvent systems

M_{153} Aq. solutions of sodium thioglycolate (0.01 - 0.2M)

M_{154} Aq. HCl and Aq. HCl - ammonium chloride mixtures

M_{155} 0.01 - 1.0M citric acid

M_{156} Aq. ammonium nitrate

M_{157} Solutions of acids , bases and salts

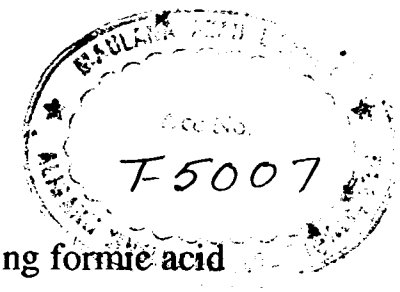
M_{158} 1.0M inorganic salt solutions in Aq. methanol

M_{159} DMSO - 1.0M HNO_3 (1+1) ; DMSO - THF (1+10) ; n - butanol-acetone - HNO_3 (6+6+1) ; diisopropyl ether - DMSO (10+1) and DMSO- THF - diisopropyl ether systems

M_{160} Several Aq. mobile phases

M_{161} Acetylacetone - acetone - conc. HCl (5+5+1)

M_{162} Distilled water



M_{163} Mixed acidic - organic solvent systems containing formic acid

M_{164} Acetone - DMSO or formic acid , acetone - DMSO - formic acid and acetone - mineral acid mixtures

M_{165} Ethanol - isobutanol - conc. HCl - water (12+6+1+1)

M_{166} 0.001 - 0.1M Aq. solutions of succinic acid

M_{167} Sixteen different solvent systems

M_{168} n- Butanol - benzene - 1M HNO_3 - 1M HCl (75+69+4+2 v/v) or acetone - 3M HCl (99+1 v/v)

M_{169} Aq. sulfuric acid and sulfuric acid - ammonium sulfate media

M_{170} 0.1M $H_2C_2O_4$, 2M NH_4Cl ,5.0M HCl , 0.5 M ammonium citrate

M_{171} DMSO - HNO_3 and DMSO - HCl systems

M_{172} Sulfuric acid , sulfuric acid - ammonium sulfate mixture

M_{173} HNO_3 at different concentrations

M_{174} Four mobile phases

M_{175} Mixtures of DMF and HNO_3 or HCl

M_{176} Aq. MeOH containing tributylphosphate and formic acid

M_{177} Sulfuric acid and organic solvents

M_{178} Acetone - chloroform (3+1)

M_{179} Formic acid and sodium formate

M_{180} MeOH - AcOH (100+0.3 v/v)

M_{181} Benzene - acetone - DMF (100:80:20)

M_{182} Mixture HCl - acetylacetone

M_{183} IBMK- formic acid

M_{184} NH_4Cl solution

M_{185} Formic acid (1.0M) ; HCOONa (1.0 M) and their mixture

M_{186} HCOONa (1.0 M) - KI (1.0 M) in the ratio 1:9

M_{187} Pyridine - benzene - HOAc - H_2O (6:5:8:4 , 5:5:4:1); BuOH - ben zene - formic acid (5:10:9)

M_{188} Formic acid (1.0 M) ; HCOONa (1.0 M) ; Double distilled water ;

HCl (1.0 M) - acetone (1:9)

M_{189} Water - in - oil microemulsion

M_{190} 15 solvent systems including NH_4OH (0.5 M)

M_{191} Aq. or organic solvents of different pH

M_{192} NH_4OH (1.0 M) - acetone (1:9, 3:7, 1:1, 7:3, 9:1)

REFERENCES

1. U.A. Th.Brinkman, G.De. Vries and R. Kuroda, *J.Chromatogr*. 85 (1973) 187 .
2. R. Kuroda and M.P. Volynets, in *CRC Handbook of Chromatography : Inorganic* ,Volume I, M. Quareshi (Ed.) , CRC press, Boca Raton , Florida (1987)89 .
3. A.Mohammad and K.G. Varshney, in *Handbook of Thin- Layer Chromatography; Inorganic and Organometallics* , J. Sherma and B.Fried (Eds.) Marcel Dekker , NY (1991) 463 .
4. A. Mohammad , in *Handbook of Chromatography* , 2nd edn., J. Sherma and B. Fried (Eds.) , Marcel Dekker ,NY (1996) 507 .
5. J. Sherma , *J. Assoc. Off. Anal. Chem.* 77 (1994) 297.
6. M.L. Langhorst , *J. Planar Chromatogr* . 2 (1989) 346 .
7. A. Mohammad , N. Fatima , J. Ahmad and M.A.M. Khan , *J.Chromatogr* . 642 (1993) 445 .
8. C.F. Poole and S.K. Poole , *Anal. Chem.* 66 (1994) 27A .
9. J. Sherma , *Anal. Chem.* 64 (1992) 134R.
10. J. Sherma, *Anal. Chem.* 62 (1990) 371R .
11. A. Mohammad and M.A.M. Khan ,*Chem. Environ. Res.* 1 (1992)3 .
12. J. Sherma and B. Fried (Eds.) , *Handbook of Thin- Layer Chromatography* , 2nd edn. Marcel Dekker , NY (1991) .
13. B. Fried and J. Sherma (Eds.) , *Thin- Layer Chromatography: Techniques and Applications* , 3rd edn. , Marcel Dekker ,NY (1994)
14. A.M. Siouffi ,*J.Chromatogr* . 556 (1991) 81 .

15. C.F. Poole and S.K. Poole , *Thin -Layer Chromatography* , in *Chromatography Today* , Elsevier , New York (1991) .

16. N.Grinberg (Ed.) , *Modern Thin - Layer Chromatography* , Marcel Dekker , New York (1990) .

17. K. Bauer , L.Gros and W. Sauer , *Thin -Layer Chromatography: An Introduction* ", EM Science , Darmstadt

18. J.C. Touchstone ,*Practice of Thin - Layer Chroamtography* , 3rd edn. ,Wiley - Interscience , New York (1992) .

19. Sz. Nyiredy and G. Szepesi ,*J. Farm. Biomed . Anal.*10 (1992) 1017.

20. Camag TLC Catalogue, Camag Scientific Co., Wilmington , NC (1988-1994) .

21. H.Ohrui , *Bunseki* 6 (1989) 470 .

22. I.V. Pletnev , E.I. Morosanova and Yu. A. Zolotov , *Anal. Sci.* 6 (1990) 863 .

23. H.S, Rathore , I. Ali , G. Sudha and T. Begum , *J. Planar Chromatogr.* 2(1989) 119 .

24. J. Gasparic , *Adv. Chromatogr.* (NY) 31 (1992) 153 .

25. I. Ojanpera , *Trends Anal. Chem.* 11(1992) 222 .

26. S. Hara ,*Kikan Kagaku Sosestu* 9 (1990) 105 .

27. H. Engelhardt ,*Naehr . Chem. Tech. Lab.* 41 (1993) 521 .

28. S. Gocan , *Chromatogr. Sci. Series* 52 (1990) 139 .

29. H.E. Hauck and W.Jost ,*Chromatogr. Sci. Series* 47 (1990) 251 .

30. Sz. Nyiredy , *J. Chromatogr. Libr.* 51A (1992) 109 .

31. E.M. Basova , T. A. Bol'shova , O. A. Shipgun and V.M. Ivanov ,*Zh. Anal. Khim.* 48 (1993) 1094 .

32. H.J. Issaq , *Trends Anal. Chem.* 9 (1990) 36 .

33. K. Robards , S. Clarke and E. Patsalides , *Analyst* 113 (1988) 1757 .

34. K. Oguma and K.Ishida , *Bunseki* 7 (1989) 549 .

35. J. Sherma ,*Anal. Chem.* 66 (1994) 67 R.

36. E. Heftmann , *Chromatography* , 5th edn. , Elsevier Science Publishers , Amsterdam , The Netherlands (1993) .

37. K. Jung and H. Specker , *Fresenius Z. Anal. Chem.* 30 (1980) 15 .

38. M.M. Senyvavin , V.A. Shulga and R.N. Rubinstien , *Zh. Anal. Khim.* 35 (1980) 2392 .

39. T. Shimizu , A. Miyazaki and I. Saitoh , *Chromatographia* 13 (1980) 119 .

40. L.B. Pfendt , M.M. Krunz and T.J. Janjic , *Microchem. Acta* 1 (1980) 385 .

41. H.G. Linde , *J. Forensic Sci.* 25 (1980) 870 .

42. P. Khanna and P.B. Chakrawarti , *J. Indian Chem. Soc.* 57 (1980) 586 .

43. M. Qureshi , B.M. Sethi and S. D. Sharma ,*Sep. Sci. Technol.* 15 (1980) 1685 .

44. K. Jung , H. Specker and I. Yenmez, *Chem. Acta Turc.* 8 (1980) 179.

45. S.P. Srivastava , K. Gupta and V.K. Dua , *J.Liq. Chromatogr.* 3 (1980) 1921 .

46. A. Longo , J.Z. Netto , L.W. Hanai and G.S. Jardin , *An. Far. Quim. (Sao Paulo)* 20 (1980) 230 .

47. A.K. De and B.K. Pal , *Sep. Sci. Technol.* 15 (1980) 1271 .

48. R.Gallago,J.L.Bernal and N.S.Perez-Franeous *An.Quim.Ser.*16(1980)408

49. J. Boodts ,C. Francoire and R. Maria , *Ciene Cult. (Sao Paulo)* 32 (1980) 201 .

50. A.K. De and P. Chakraborty ,*J. Liq. Chromatogr.* 4 (1980) 2223 .

51. A.K. Sen and U.C. Ghose ,*J. Liq. Chromatogr.* 3 (1980) 71 .

52. J. Franc and E. Kosikova ,*J. Chromatogr.* 187 (1980) 462 .

53. H.M. Chawla , N.N. Ralhan ,N.K. Garg and S.S. Chibbar , *J. High Resolut. Chromatogr. Chromatogr. Commun.* 3 (1980) 651 .

54. K. Kawanabe and K. Maruyama , *Meiji Yakka Diagaku Kenkykiyo* (1980) 25 .

55. D. Lucansky , V. Batora , J. Teren , V. Nigrovicova and Z. Kohut , *Agrochimia* 20 (1980) 299 .

56. T. Morita , T. Hamada and K. Ishida , *Fresenius' Z. Anal. Chem.* 309 (1981) 377 .

57. S. Gocan and C. Costea , *Babes - Bolyai (Ser.) Chem.* 26 (1981) 31 .

58. K. Ishida , T. Morita and T. Hamada ,Fresenius' *Z. Anal. Chem.* 305 (1981) 257 .

59. H. Specker ,*Chem. Labor . Betr.* 32 (1981)519 .

60. R. Kuroda and N. Hosoi ,*Chromatographia* 14 (1981) 359 .

61. H.M. Chawla , N.K. Garg and S.S. Chibbar , *J. High Resolut Chromatogr. Chromatogr. Commun* . 4 (1981) 657 .

62. I. Yenmez , *Chim. Acta Turc.* 9(1981) 367 .

63. P. Kitaeva and M.P. Volynets ,*Zh. Anal. Khim.* 36 (1981) 1490 .

64. S.S. Joshi , R.H. Harider and R.K. Srivastava , *J. Indian Chem. Soc.* 58 (1981) 193 .

65. K. Ravindranath and P.B.Janardhan , *Indian J.Chem.* 20 A(1981)282.

66. N.V.N.Kumar and Y.P.Devi, *J. Assoc.Off. Anal.Chem.* 65 (1981) 729.

67. Y.P.Devi and N.V.N.Kumar,*J. Assoc.Off. Anal.Chem.* 64(1981)1301.

68. J.A. Stratis ,I.N. Papadoyannis and G.S. Vasilikiotis , *Microchem.J.* 26 (1981) 40 .

69. K. Kawanabe and K. Maruyama , *Yakugaku Zashi* 101 (1981) 912 .

70. T.Shimizu ,H.Tanaka and T.Ohsawa ,*Chromatographia*15 (1982)177.

71. T. Nagayama , M. Nishijima , H. Kamimura , K. Yasuoda , I.Akihiro , S. Kazuo , H. Ushiyama and N. Yasuta , *Shokuhim Eiseigaku Zasshi* 23 (1982) 253 .

72. K. Ravindranath and P.B. Janardhan ,*Indian J.Chem.* 21 A(1982)150.

73. G.V. Alexeeff , P.Munoz and W.W. Kilgore ,*J. Agric. Food Chem.* 30 (1982) 780 .

74. N.S. Seth and R.P.S. Rajput , *Indian J. Chem.* 22 A (1983) 1088 .

75. M. Qureshi , B. M. Sethi and S.D. Sharma , *J. Liq. Chromatogr.* 6 (1983) 1249 .

76. G. Buchbauer and J. Knie , *Sci. Pharm.* 51 (1983) 41 .

77. T. Hamada, T. Morita and K. Ishida , *Fresenius ' Z. Anal. Chem.* 316 (1983) 23.

78. D.S. Gaibakyan , J.K. Rozylo and M. Poniewaz , *J.Phys. Chem.* 38 (1983) 1 .

79. N. Perisic-Janjic , V. Canic and S. Radosavljevic , *Chromatographia* 17 (1983) 454 .

80. Y.Chen, K.Zheng, H.Luo and J.Cheng, *Fenxi Huaxue* 11 (1983) 101.

81. Le Van So, *Radioanal. Lett.* 59(1983)53.

82. S.D. Sharma , T.R. Sharma and B.M. Sethi , *J. Liq. Chromatogr.* 6 (1983) 1253 .

83. S.P. Srivastava , K. Gupta and V.K. Gupta , *J.Liq. Chromatogr.* 6 (1983)145 .

84. J. Franc , *Czech . C S 210 119 (Cl. G 01/N31/08) 15 July 1982 Appl. 79/3, 566, 24 May (1979)* , *Chem. Abs.* 98 (1983) 154643 x .

85. K. Taguchi , K. Ogata , K. Tanaka ,S. Tanabe and T. Imanari , *Bunseki Kagaku* 32 (1983) 20 .

86. T.N. Galkava , L.I. Petrovskay , I.L. Shoshkova and E.A. Prodan , *Zh. Anal. Khim.* 38 (1983) 1640 .

87. G. Buchbauer and R.E. Markis , *Sci. Pharm* . 51 (1983) 48 .

88. S.P. Srivastava , R. Bhushan and R.S. Chauhan , *J. Liq. Chromatogr.* 7 (1984) 1341 .

89. T. Shimizu ,K. Tadokoro ,A. Suganuma and M. Hirose , *Chromatogrphia* 18 (1984) 692 .

90. M. Qureshi , B.M. Sethi and S.D. Sharma , *J. Liq. Chromatogr.* 7 (1984) 1345 .

91. H.Specker and A.Hufnogie, *Fresenius Z.Anal.Chem.*318 (1984) 198.

92. C. Costea , *Bulg. Univ. Brasov. Ser. C.* 24 (1984) 5 .

93. H. Luo and X . Liu , *Fenxi Huaxue* 12 (1984) 1077 .

94. L.B.Pfendt, S.Petrovic and V.Z.Pasajlic, *Microchem.J.* 29 (1984)296.

95. N. Fatima and A. Mohammad, *Sep.Sci. Technol.* 19 (1984) 429 .

96. J.Z.Netto, A.F.C.Graner and M.Ionashiro,*Ecletica Quim.*9 (1984) 45.

97 I.Iordanova and D.Lolova, *Khig. Zdrareopaz* 27 (1984) 488 .

98. G. De Vries and U.A. Th. Brinkman , *Mikrochim. Acta* 2 (1984) 47 .

99. R. Bhushan ,S.P. Srivastava and R.S. Chauhan , *Anal. Lett.* 18 (1985)1549 .

100. T. Shimizu ,T. Kurosaki and H. Suwa , *Chromatographia* 20 (1985) 364 .

101. S.P. Srivastava , R. Bhushan and R.S. Chauhan , *J. Liq. Chromatogr.* 8 (1985) 571 .

102. D.S. Gaibakyan , J.K. Rozylo and M. Poniewaz , *Chem. Anal.* 30 (1985) 527 .

103. A.K.Sen , S.B. Das and U.C. Ghosh , *J. Liq. Chromatogr.* 8 (1985) 2999 .

104. M. Qureshi , A. Mohammad and N. Fatima , *J. Liq. Chromatogr.* 8 (1985) 1279 .

105. D.S. Gaibakyan , J.K. Rozylo and M. Janicka , *J.Liq. Chromatogr.* 8 (1985) 2969 .

106. W. Ding , B. Qian and C. Yang , *Zhongguo Xitu Xuebao* 3 (1985) 79.

107. H.Luo and H.Wang,*Wuhan Daxue Xuebao Ziran Kexueban*(1985)25.

108. J. Liu , Z. Yan , L. Lui and J. Cheng , *Wuhan Daxue XuebaoZiran Kexueban* (1985) 9 .

109. K.G. Varshney , A.A. Khan and S. Anwar ,*J. Liq. Chromatogr.* 8 (1985) 1347 .

110. B. Yin and J. Lui , *Huaxue Tongbao* 7 (1985) 25 .

111. J. Sherma ,A. P. Lee , M.S. Bowker and A. J. Kind ,*Tech. Appl. Thin Layer Chromatogr. (Proc. Bienn. Symp. Thin Layer Chromatography*) 1982 (Publ. 1985); *Chem. Abs.* 102 (1985) 216276 z .

112. W. Werner , Fresenius *J. Anal. Chem.* 321(1985) 374 .

113. R.P.S. Rajput and S. Agrawal , *Anal. Lett.* 18 (1985) 1783 .

114. E. Kirst , *Nahrung* 29 (1985) 391 .

115. P. Bruno , M.Caselli and A. Traini , *J. High Resolut. Chromatogr. Chromatogr. Commun* .8 (1986) 366 .

116. A. Wu , Z. Mao ,C. Chen and G. Chen, *Fenxi Huaxue* 14 (1986) 525.

117. A. Mohammad and N. Fatima , *J. Liq. Chromatogr.* 9 (1986) 1903 .

118. K. Ishida , S. Ninomiya , Y. Takeda and K.Watanabe , *J.Chromatogr.* 351 (1986) 489 .

119. M. Kastelan-Macan , Lj. Bokic , S. Cerjan-Stefanovic and K. Moska-Liuk , *Chromatographia* 22 (1986) 19 .

120. T. Simizu and S. Abe, *Chromaotgraphia* 21 (1986) 708 .

121. J. Moslowska and Z. Mlodzikowski , *Chemica Analityezna*31 (1986) 193 .

122. G.V. Myasoedova , M.P. Volynets , T.G. Akimova , L.I. Bol'Shakova, T.V. Dubrova ,S.B. Savvin and A.N. Ermakov , *Zh. Anal. Khim.* 41 (1986) 662 .

123. M. Ajmal , A. Mohammad and N. Fatima , *J. Liq. Chromatogr.* 9 (1986) 1877.

124. A. Mohammad and N.Fatima, *Chromatographia* 22 (1986) 109 .

125. T.Shimizu, E.Uehara and M.Ohtani, *Chromatographia* 21(1986)175.

126. H.Luo, R.Xie and K. Zheng , *He Huaxue Yu, Fangshe Huaxue* 8 (1986) 185 .

127. M. Lin , W. Zong , X. Jia , Zh. Hu and J. Zheng , *Huaxue Shijie* 27 (1986) 261 .

128. S. Ren , W. Zong , X. Zia, Zh. Hu and J. Zheng , *Huaxue Shijie* 27 (1986) 403 .

129. K.Ravindranath and P.B.Janardhan, *Indian J.Chem.* 25 A (1986)405.

130. T. Shimizu , H. Tanaka and M. Kobayashi , *J. Planar Chromatogr.* 8 (1986) 469 .

131. W. Vyneke , *Rev. Agric. (Brussels)* 39 (1986) 177 .

132. T.Shimizu ,Y. Suzuki and C.Inose, *Chromatographia* 23 (1987) 648.

133. L. Liu , J. Liu , Z. Hung and J. Cheng , *Wuhan Daxue Xuebao Ziran Kexueban* 1 (1987) 64 .

134. H. Luo and R. Xie , *Kexue Tongbao* 32 (1987) 1041 .

135. H. Lin and S. Yi , *Sepu* 5 (1987) 53 .

136. J. Wang and C.Hu , *Huaxue Tongbao* 6 (1987) 29 .

137. H. Luo and H. Wang , *Wuhan Daxue Xuebao Ziran Kexueban* 1 (1987) 79 .

138. O. Hadzija, M. Tonkovic and S. Iskvic, *J. Liq. Chromatogr.* 10 (1987) 3673 .

139. A.Mohammad and N. Fatima , *Chromatographia* 23 (1987) 653 .

140. A. Mohammad and N.Fatima, *J. Liq.Chromatogr.* 10(1987)1349 .

141. H.Zeng and K.Lu, *Shipin Yu Fajiao Gongye* (1987) 47 .

142. M.Ajmal, A.Mohammad and N.Fatima, *Microchem.* J.37 (1988) 314.

143. M.Ajmal, A.Mohammad , N.Fatima and J.Ahmad, *J.Planar Chromatogr* . 1(1988) 329 .

144. M.Ajmal, A.Mohammad, N.Fatima and J.Ahmad, *J.Plannar Chromatogr* . 1(1988) 239 .

145. M.Ajmal, A.Mohammad , N.Fatima and A.H.Khan , *J.Planar Chromatogr* . 1(1988) 128 .

146. A.Mohammad and N.Fatima, *Chromatographia* 25(1988) 536 .

147. H.Luo and J.Mo, *Fenxi Huaxue* 16 (1988) 821 .

148. J.Wang and Zh. Hu, *Huaxue Shijie* 10(1988) 136 .

149. H.Luo and J.Mo, *Fenxi Shyanshi* 7(1988) 27 .

150. G.Cheng , Zh. Hu, X.Jia and J.Zheng, *Fenxi Huaxue* 16 (1988)1098.

151. J.Wang, Zh. Hu and J.Zheng, *Yankuang Ceshi* 7(1988) 10 .

152. R.Kuroda, M.Adachi and K.Oguma,*Chromatographia* 25(1988) 989.

153. R.Kuroda , S. Ishimaru and K.Oguma, *Anal. Sci.* 4(1988) 667 .

154. C.Glidewell and J.S.McKechnie, *J.Chem. Educ.* 65(1988) 1015 .

155. R.P.S. Rajput, A.K. Misra and S. Agrawal , *J.Planar Chromatogr* . 1(1988) 349 .

156. S.Ninomiya, N.Takeda and K.Ishida, *Fresenius' Z.Anal.Chem.* 332 (1988) 798 .

157. G.Cheng, Zh. Hu, X.Jia and J.Zheng , *Sepu* 6 (1988) 108 .

158. J.Wang and Zh. Hu, *Fenxi Huaxue* 16 (1988) 740 .

159. S.Ren, Zh. Hu, X.Jia and J.Zheng, *Sepu* 6 (1988) 253 .

160. A.K.Misra and R.P.S. Rajput , *J.Liq. Chromatogr.* 11 (1988) 3145 .

161. Y.Ma and E.S.Yeing, *Anal. Chem* .60 (1988) 729 .

162. H.Luo and J.Xi,*Wuhan Daxue Xuebao Ziran Kexueban* 4(1989)125.

163. M.Ajmal, A.Mohammad and N.Fatima, *Indian J.Chem.* 28 A(1989) 91 .

164. L.Deshmukh and R.B.Kharat, *J.Liq. Chromatogr.* 12(1989) 937 .

165. L.Deshmukh and R.B.Kharat, *Int. J. Environ. Anal.Chem.*16(1989)1.

166. T.Shimizu , N.Arikawa and K.Novaka, *J.Planar Chromatogr .* 2 (1989) 90 .

167. T.Shimizu, H.Hirata and K.Nakajima , *Chromatographia* 28 (1989) 620 .

168. T.Shimizu , K.Nonaka and N.Arikawa , *J.Planar Chromatogr .* 2 (1989) 393 .

169. X.Jin, M.Zhang, F.Wu, Y.Zhang and X.Peng, *Yejin Fenxi* 9 (1989)49.

170. A. Mohammad , M.Ajmal , N.Fatima and J. Ahmad , *J.Liq. Chromatogr.* 12 (1989) 3163 .

171. M. Ajmal , A. Mohammad , N. Fatima and A.H. Khan, *Microchem.J.* 39 (1989) 361 .

172. S.W.Hussain and V.Ghoulipour, *J. Planar Chromatogr.* 2 (1989) 474.

173. L. Gao, J.Fan , Y.Yu and S.Wang, *Shandong Daxue Xuebao, Ziran Kexueban* 24 (1989) 69 .

174. G.Cheng, Zh. Hu and X. Jia , *Fenxi Cashi Tongbao* 8 (1989) 55.

175. E.I. Polyanebiskii, S.A. Butova , V.A. Matii and V.A. Shandro, *Vich. Obogashch Rud (Leningrad)* 3 (1989) 32 .

176. S. Ren , Zh. Hu, S.Jia and J. Zheng , *Huaxue Shijie* 11 (1989) 367.

177. B. Gawdzic, D.S. Gaibakyan , T. Matynia and A.R. Sarkisyan . *J. Planar Chromatogr.* 3 (1990) 280 .

178. J.K. Razylo , D. Gwischomicz and I. Malinowska , *Environ. Protect. Eng.* 16 (1990) 121 ; *Chem. Abs.* 121 (1994) 260038 m .

179. L. Deshmukh and R.B.Kharat , *J. Chromatogr. Sci.* 26 (1990) 400 .

180. K.Kumari and R.P. Singh , *J. Planar Chromatogr.* 3 (1990) 349 .

181. L.Deshmukh , P.D. Kichambare and R.B. Kharat , *J. Indian Chem. Soc.* 67 (1990) 612 .

182. I. Malinowska, J.K. Rozylo and D.S. Gaibakyan, *J. Planar Chromatogr.* 3 (1990) 422 .

183. Q. Lei and Y.Li, *Fenxi Ceshi Tongbao* 9 (1990) 22 .

184. A.K. Misra and R.P.S. Rajput , *J. Planar Chromatogr.* 3 (1990) 83 .

185. A.Jain , O.V. Singh and S.N. Tandon , *J. Planar Chromatogr.* 3 (1990) 79 .

186. T. Simizu, T.Ohtomo and T. Shimizu , *J. Planar Chromatogr.* 3(1990) 88 .

187. V.A. Ryabutchin , M.P. Volynets and B.F. Myasoedor ,*Zh. Anal. Khim.* 45 (1990) 279 .

188. J.K. Rozylo, I. Malinowska and D.S. Gaibakyan , *J. Planar Chromatogr.* 3 (1990) 157 .

189. K. Ishida, Y. Uchida , S. Ninomiya and M. Osawa , *Fresenius J. Anal. Chem.* 336 (1990) 419.

190. T.Hamada ,T.Morita, M.Matsuzuka and K.Ishida , *Fresenius J.Anal.Chem.* 338 (1990) 54 .

191. K.S.Panesar,O.V.Singh and S.N.Tandon, *Anal. Lett.* 23 (1990) 125.

192. I.Skvorc, S.Iskric, N. Zambeli and O.Hadzija, *J. Chromatogr.* 498 (1990) 428.

193. A. Mohammad , M. Ajmal , N.Fatima and J. Ahmad , *J. Planar Chromatogr.* 3 (1990) 396 .

194. M.L. Liao and P.A. Seib , *J. Agric. Food Chem.* 38 (1990) 355 .

195. A. Mohammad and S.Tiwari , *Chromatogrphia* 30 (1990) 405 .

196. T. Shimizu , R.Igarashi , Y. Hayashi and S. Jindo , *J. Planar Chromatogr.* 4 (1991) 487 .

197. T. Shimizu , K. Hashimoto and K. Tsunoda, *Chromatograpia* 31 (1991) 60 .

198. K.Ishida , S. Ninomiya , Y. Uchida and M. Osawa *J. Chromatogr.* 539 (1991) 169 .

199. N.U. Perisic-Janjic , S.N. Petrovic and S. Podunavoc, *Chromatographia* 31 (1991) 281 .

200. L.Deshmukh and R.B.Kharat, *J. Planar Chromatogr.* 14 (1991)1483.

201. T.Shimizu, S.Jindo and D.Kanoh, *J.Planar Chromatogr.* 4(1991)261.

202. A. Jain , O.V. Singh and S.N. Tandon ,*Indian J. Chem.* 30 (A) (1991) 196 .

203. N.D. Betenekov ,G. V. Buklanov , E.G. Ipatova and Yu. S.Korotkin, *Radiokhimiya* 33 (1991) 163 .

204. S.E.Gasparyan and V. A. Sarkisyan, *Arm. Khim . Zh.* 44(1991) 417 .

205. J.K. Rozylo , D.Gwischomicz and I.Malinowska , *Chem. Anal. (Warsaw)* 36 (1991) 279 .

206. S.D. Sharma and S.Misra , *J. Liq. Chromatogr.* 14 (1991) 3301 .

207. I. Malinowska and J. K.Rozylo, *J.Planar Chromatogr.* 4 (1991) 138 .

208. J.Z. Netto , *Ecletica Quim.* 16 (1991) 9 .

209. A. Mohammad and S. Tiwari *J. Planar Chromatogr.* 4 (1991) 485 .

210. A. Mohammad ,M.Ajmal , N. Fatima and J. Ahmad ,*J. Liq. Chromatogr.* 14 (1991) 3283 .

211. A.K. Misra and R.P.S. Rajput , *Proc. Natl. Acad. Sci. (India) Sect.* A 61 (1991) 469 .

212. A.Mohammad and S. Tiwari , *Microchem. J.* 44(1991) 39 .

213. Lj. Bokic , M. Petrovic , M. Kastelan- Macan and K. Moskalinte, *Chromatographia* 34 (1992) 648 .

214. J.H. Aldstadt and H.D.Dewald , *Anal. Chem.* 64 (1992) 3176 .

215. K.S. Panesar , O.V. Singh and S.N. Tandon ,*J. Planar Chromatogr.* 5 (1992) 289 .

216. S. Przeszlakomski and M.Maliszenska , *Chem. Anal. (Warsaw)* 37 (1992) 545 .

217. A. Mohammad , M.Ajmal, N.Fatima and M.A.M. Khan , *J.Planar Chromatogr.* 5 (1992) 368 .

218. M. Yoshioka , H. Araki, M.Seki, T.Miyazaki , T.Utsuki , T.Yaginuma and M. Nakano , *J. Chromatogr.* 603 (1992) 223 .

219. T. Shimizu ,F.Nishimura , T.Imai and S. Jindo , *Chem. Environ Res.* 1 (1992) 73 .

220. E. I. Morosanova , L.S.Seliveltrova and Yu. A.Zolotov , *Zh. Anal. Khim.* 48 (1993) 617 .

221. V.Sanchez-Camazano and M.J.Sanchezmartin , *J.Chromatogr.* 643 (1993) 357 .

222. N.M.Kuznin ,V.M.Pukhovskaya , G.N. Varshed , B.Ya. Spivakov , N.A.Maryutina , M.P.Volynets , V.A.Ryabukhim ,N.N.Chphitiya , O.N.Goebneva and Y.M.Paututskaya , *Zh.Anal.Khim.* 48 (1993) 898.

223. S.D.Sharma ,S.Misra and R. Gupta,*J.Liq.Chromatogr.*16(1993)1833.

224. C. Z.Soljic and Z.Hrestrk,*Khim.Ind.* 42 (1993) 359.

225. K. G.Varshney ,A .A.Khan and M.S. Siddiqi, *Proc.Natl Acad. Sci., India, Sect.* A 63 (1993) 495.

226. M.Joanna and O.Alina ,*Przem.Chem.* 72(1993)495.

227. A.Mohammad and S.Tiwari ,*Microchem. J.*47 (1993) 379.

228. O.Shadrin ,V.Zhivopistsev and A. Timberbaev,*Chromatographia* 35(1993) 667 .

229. T.Shimizu , S.Jindo, N.Iwata and Y.Tamura, *J. Planar Chromatogr.* 7 (1994) 98 .

230. S.K.Yadav, O.V.Singh and S.N.Tandon, *J.Planar Chromatogr.* 7 (1994) 75 .

231. S.A.Nabi, W.U.Farooqui, and Rehman, *J.Planar Chromatogr.* 7 (1994) 36 .

232. A. Mohammad, M.Ajmal, N.Fatima and R.Yousuf , *J.Planar Chromatogr.* 7(1994) 444 .

233. T.Shimizu, S.Jindo, M.Saitoh and Y.Mura, *J.Planar Chromatogr.* 7(1994) 412 .

234. A.Mohammad,N.Fatima and M.A.M. Khan, *J.Planar Chromatogr.* 7 (1994) 142 .

235. D.M.Jogadankar, V.B. Patil and H.N.Katkar, *J.Planar Chromatogr.* 7 (1994) 166 .

236. M.E.M.Hassouna, S.M.Sayyah, El-Salam and H.M.Abd, *Anal. Lett.* 27(1994) 2535 .

237. Y.Jianjin, *Gansu Gongye Daxue Xuebao* 20 (1994) 102 .

238. Y.Jianjin, *Gansu Gongye Daxue Xuebao* 21 (1995) 104 .

239. C.Z.Soljic, Z.Hrestak and I.Eskinja, *Kem. Ind.* 44 (1995) 219 .

240. Y.Takeda, T.Nagai and K.Ishida, *Fresenius' J.Anal. Chem.* 351 (1995) 186 .

241. N.P.Molochnilcova, V.M.Shkinev and B.F. Myasoedova, *Radiokhimiya* 37 (1995) 517 .

242. A.Mohammad and M.A.M.Khan, *J.Planar Chromatogr.* 8(1995)134.

243. A.Mohammad , *J.Planar Chromatogr.* 8 (1995) 463.

244. V.K.Gupta, I.Ali, U.Khurana and N.Dhaggara, *J.Liq. Chromatogr.* 18(1995) 1671 .

245. A.Mohammad, S.Tiwari and J.P.S.Chahar, *J.Chromatogr. Sci.* 33(1995) 143 .

246.A.Mohammad, S.Tiwari, J.P.S.Chahar and S.Kumar, *J.Am. Oil Chem. Soc.* 72 (1995) 1533 .

247. S.W. Hussain, A. Avanes and V.Ghoulipour, *J.Planar Chromatogr.* 9 (1996) 67 .

248. S.C.Petrovic and H.D.Dewald, *J.Planar Chromatogr.* 9 (1996) 269 .

249. I.Baranowska, J.Baranowski, I.Norska-Borowska and C.Pieszko, *J.Chromatogr.* A 725 (1996) 199 .

250. S.Savasci and M.Akay, *Turk. J.Chem.* 20 (1996) 146 .

251. K.Yoshimura,T.Horita and K.Hozuni,*Polym.J. (Tokyo)* 28 (1996)261.

252. S.Wang, D.Xi and J.Li, *Yejin Fenxi* 16 (1996) 12 .

253. S.B.Germanov, L.P.Sharova, T.F.Demchuk, N.I.Damoilenko and F.V.Guss, *Khim. Farm. Zh.* 30 (1996) 54 .

254. A.Mohammad and J.P.S.Chahar, *J.Chromatogr. A* 774 (1997) 373 .

CHAPTER -III

THIN LAYER CHROMATOGRAPHIC SEPARATION, IDENTIFICATION AND DETERMINATION OF CERTAIN ANIONS

3.1 INTRODUCTION

Of the various chromatographic techniques, thin- layer chromatography (TLC) has recently gained popularity not only because of advancements in technique and instrumentation and improvement in efficiency but also because of its relatively low cost and speed of analysis compaired with other highly selective and efficient chromatographic techniques such as high performance liquid chromatography and gas chromatography . The differential migration of species in TLC is due to varying degrees of affinity of the components in the stationary and mobile phase mixtures .The exact separation mechanisms involved depend on the nature of the two phases and the solutes .Chromatographic retention and selectivity depend upon interactions such as hydrogen bonding and electron pair donor and acceptor, ion-ion, ion-dipole, and van der Waals interactions.

As extensive literature survey (1-10) on TLC was undertaken ; it is surprising to note that very little work has been reported on the analysis of anionic mixtures as compared with that of cationic mixtures. As a continuation of our previous work on the TLC of anions (11-13) , this chapter describes a simple method for identification, separation, and determination of I^-, SCN^-, NO_2^-, Br^-, BrO_3^-, IO_4^-, IO_3^-, CrO_4^{2-}, and PO_4^{3-} under various experimental conditions.

3.2 EXPERIMENTAL

***Apparatus*:** A TLC applicator (Toshniwal, India) was used for prepration of 20×3.5 cm glass plates . The chromatography was performed in 24×6 cm glass jars.

***Chemicals*:** Silica gel G (particle size between 10 and 40 μm; Catalog No.

27335) and methanol were obtained from Glaxo Laboratories (India) . kieselguhr (particle size between 10 and 40 μm; Catalog No. 015037) , kaolin (Catalog No. 033059) and cellulose microcrystalline (particle size less than 30 μm; Catalog No. 027984) were obtained from CDH Laboratories (India). All other reagents were of Analytical Reagent grade .

***Test Solutions*:** The test solutions (1% w/v) were sodium salts of nitrate, nitrite ; fluoride and potassium salts of iodide, iodate, periodate, bromide, bromate, permanganate, chromate, dichromate, ferricyanide, ferrocyanide, chloride, phosphate, and sulfite ; thiocyanate and molybdate were taken as their ammonium salts. Double distilled water with a specific conductivity(K= 2×10^{-6} ohm^{-1} cm^{-1} at 25°C) was used for the preparation of salt solutions.

Detection Reagents :For the detection of various anions, the following reagents were used:

(a) Saturated $AgNO_3$ solution in the methanol for I^- , Br^-, Cl^-, F^-,SO_3^- $Cr_2O_7^{2-}$, CrO_4^{2-} and PO_4^{3-} .

(b)Diphenylamine (0.2-0.5%) in 4M H_2SO_4 for NO_2^-, NO_3^-,IO_4^-, IO_3^-, BrO_3^-, MnO_4^- and WO_4^{2-}.

(c) Ferric chloride (10%) in 2.0M HCl for SCN^-, $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$.

(d) Alcoholic pyrogallol (0.5%) for MoO_4^{2-} and $Mo_7O_{24}^{6-}$.

***Mobile Phases*:** The following solvents were investigated: M_1 , 0.1M HCl-acetone (1:9); M_2,1.0M formic acid ; M_3 , 1.0 M sodium formate ; M_4 ,double distilled water .

Stationary Phases : The following sorbent layers were used : S_1 , silica

gel impregnated with aqueous 1% $CuSO_4$ solution ; S_2 , silica gel G ; S_3 , alumina ; S_4 , cellulose microcrystalline ; S_5 , kaolin ; S_6 kieselguhr G; S_7, alumina + cellulose (2:1); S_8 , kieselguhr + cellulose (1:2,2:1) .

Procedure :

(a)*Preparation of TLC plates*: Silica gel, alumina , and kieselguhr TLC plates were prepared by mixing the adsorbent with double distilled water in a 1:3 ratio by weight. The resultant slurry was mechanically shaken for 10 min after which it was applied to well-cleaned glass plates with the help of TLC applicator to give a layer of approximately 0.25 mm thickness. The plates were air dried at room temperature and then heated at 100± 5^{0}C for 1 h to activate them . After activation, the plates were stored in a desiccator. Cellulose or kaolin coated plates were prepared in a similar fashion by using a slurry made by mixing cellulose or kaolin with double distilled water in a 1:4 ratio by weight. No additional binder was added to the slurry. For impregnated silica gel layers, a slurry was made by mixing silica gel with an aqueous solution of 1% $CuSO_4$ in a 1:3 ratio . Thin layers were then prepared as described previously .

Qualitative analysis : A sample volume (1.0-10 µL) containing a sufficient amount of analyte (0.1-10 µg) was applied using a micropipette about 2.0 cm above the lower edge of the chromatoplates . The spots were dried , and the plates were developed in glass jars containing the mobile phase using a one- dimensional ascending technique . Before developing the plates, the glass jars that contained the mobile phase were covered with a lid for about 20 min so that the glass jars would get pre-saturated with the mobile phase vapors .

The mobile phase (solvent) was allowed to migrate up to 10 cm from the starting line in all cases . After development , the plates were dried at room temperature and the anion spots were visualized using the appropriate spray reagent (Figure 3.2) .

Quantitation : For semi-quantitative determination of I^- , Br^- and NO_2^- , 0.01 ml of various standard solutions of KI , KBr and $NaNO_2$ (2.5-40%) were spotted on silica gel layers impregnated with a 1% $CuSO_4$ solution.The chromatoplates were developed with M_1 . After detection, the spot was copied onto tracing paper from the chromatoplate and then the spot-area was calculated graphically of triplicate tests .

The limit of detection of various anions was determined by spotting different amounts of anionic solutions on the chromatoplates . The plates were developed and the spots were detected as described above . The method was repeated with successive lowering of the amount of anion until spots could no longer be detected . The minimum amount of anion that was able to be detected was taken as the limit of detection .

A volumetric procedure was applied for the quantitative determination of iodate after its TLC separation from periodate . A standard volumetric method (14) was set up using 0.01M sodium thiosulfate solution as an intermediate solution . For the determination of IO_3^- in the presence of IO_4^- various samples containing a mixture of KIO_3 and KIO_4 in variable amounts were prepared . Using a lambda pipette , 0.01 ml of the resultant mixture was loaded on the chromatoplates . The plates were developed with double distilled water (15) . A pilot plate was employed simultaneously in order to locate the exact position of the spot on the working plate . The area

occupied by IO_3^- was scraped and IO_3^- was extracted with 1.0M HCl .The adsorbent was separated from the solution by filtration and washed with 1.0 M HCl to ensure complete extraction of IO_3^- . The filtrate was added to a blank that was prepared by mixing 2 ml KIO_3 (1%) . The contents were titrated with a 0.01 M $Na_2S_2O_3$ solution , the blank was also simultaneously titrated with 0.01M sodium thiosulfate and the difference between the volume of the $Na_2S_2O_3$ solution consumed in both cases was taken for the determination of IO_3^- in the sample .

3.3 RESULTS AND DISCUSSION

Tables 3.1 and 3.2 show that the chromatographic systems can be successfully applied for the separation of several anions from their binary and ternary mixtures . The results in Table 3.1 indicate that well resolved ternary separations of IO_4^- and IO_3^- can be realized from a synthetic mixture containing I^- or SCN^- and NO_2^- , Br^- , BrO_3^- ,CrO_4^{2-} or PO_4^{3-} . The proposed method is well - suited for microgram detection of anions on cellulose microcrystalline layers . It is clear from Table 3.2 that distilled water can also be used as a non-toxic eluant for some binary separations of anions. In addition to qualitative separations , quantitative and semi-quantitative determination of certain anions with preliminary separation on thin layers can also be made .Table 3.3 presents the results of quantitative determination of iodate in the presence of periodate . It is evident from Table 3.3 that the proposed method is accurate (percent error, $\pm$ 2.2) and reproducible (relative standard deviation , 18 ppt).

We attempted to semiquantitatively determine IO_4^- ,IO_3^- , NO_2^- ,Br^- , and I^- by measuring the size of the spots . We outlined the spots on a piece of

Table 3.1 R_F Ranges (sample size, n=3) Involved in Ternary Separation on Cellulose Microcrystalline Thin Layers Using 0.1 M HCl - Acetone (1:9) as Eluant*

Anions Separated	Lower detection limit (μg)	R_F	Accompanying anions
I^-	7.64	0.66-0.75	IO_4^-, IO_3^-, NO_2^-, Br^-, BrO_3^- PO_4^{3-}, CrO_4^{2-}
SCN^-	3.81	0.73-0.78	IO_4^-, IO_3^-, NO_2^-, BrO_3^-, PO_4^{3-}, CrO_4^{2-}
NO_2^-	3.33	0.35-0.41	IO_4^-, IO_3^-, SCN^-, Br^-, I^-
Br^-	6.71	0.37-0.38	IO_4^-, IO_3^-, I^-
BrO_3^-	3.83	0.27-0.32	IO_4^-, IO_3^-, SCN^-, I^-
PO_4^{3-}	11.58	0.32-0.38	IO_4^-, IO_3^-, SCN^-, I^-
CrO_4^{2-}	4.06	0.35-0.42	IO_4^-, SCN^-, Br^-, I^-
IO_4^-	4.15	0.04-0.06	I^-, SCN^-, NO_2^-, Br^-, BrO_3^-, PO_4^{3-}, CrO_4^{2-}
IO_3^-	4.08	0.04-0.07	I^-, SCN^-, NO_2^-, Br^-, BrO_3^-, PO_4^{3-}

**Standard R_F values of individual ions: I^-, 0.73; SCN^-, 0.83; NO_2^-, 0.41; Br^-, 0.45; BrO_3^-, 0.40; IO_4^-, 0.07; IO_3^-, 0.08; CrO_4^{2-}, 0.74; PO_4^{3-}, 0.43.*

Table 3.2 : Separations Achieved Experimentally on Different Sorbent Layers Using Distilled Water as Eluant

Sorbent system	Separation(R_L-R_T)*
Silica gel 'G'	IO_4^-(0.00)-IO_3^-,BrO_3^-,NO_2^-,I^-,WO_4^{2-}, $Fe(CN)_6^{3-}$,$Fe(CN)_6^{4-}$(1.0-0.85)
Alumina	NO_3^-(1.0-0.82) -WO_4^{2-},MnO_4^-,PO_4^{3-}(0.00), NO_3^-(1.0-0.82) - CrO_4^{2-} or $Cr_2O_7^{2-}$ (0.18-0.00) NO_3^-(1.0-0.86) - $Fe(CN)_6^{3-}$(0.1-0.00) SCN^-(1.0-0.86) - CrO_4^{2-} or $Cr_2O_7^{2-}$ (0.19-0.00)
Cellulose	MnO_4^- (0.00) - IO_4^-, IO_3^-, CrO_4^{2-} or $Cr_2O_7^{2-}$(1.0-0.90)
Alumina + Cellulose (2:1)	MnO_4^-(0.00- 0.00)-IO_3^-(0.45- 0.30)-NO_2^- (1.0-0.89) MnO_4^- (0.00 - 0.00) -IO_3^-(0.41 -0.24) -BrO_3^- (1.0- 0.86)

R_L is the R_F of the leading front and R_T is the R_F of the trailing front of the spot .

Table 3.3: Determination of Iodate with Preliminary TLC Separation from Periodate on Silica Gel G Layers with Distilled Water as the Eluant

Amount of IO_4^- taken (mg)	Amount of IO_3^- loaded (mg)	Amount of IO_3^- recovered* (mg)	Error (%)	Relative standard deviation (ppt)
0.415	0.405	0.412	- 1.7	18.1634
0.365	0.450	0.441	+ 2.0	15.3794
0.307	0.500	0.489	+ 2.2	13.0410
0.232	0.590	0.601	- 1.9	12.6718

**Each value is the average of five determination (n= 5)*

paper and determined the weight of the paper strip covering the spot-area . A linear realationship was obtained when the weight of the paper covering the spot-area was plotted against the area of the spot (Figure3.2) . The linearity was maintained up to 160 mg of NO_2^- , 80 mg of I^- , and 100 mg of Br^- . The deviation from linearity started if the loading amount exceeded the upper limit . The reproducibility of data plotted in figure 3.2 was checked by two independent analysts . The values obtained by both the analysts differ by ± 15% from the average value as plotted in Figure 3.2 . Thus , the method can safely be applied for semi-quantitative determination of Br^- ,I^- and NO_2^- . However , the semi-quantitative method was unsuitable for IO_3^- and IO_4^- because of the lower solubility of KIO_3 or KIO_4 in water .

A plot of loading amount versus spot-area for NO_2^-,Br^- , and I^- follows the equation y = mx + c , where c has positive values . However , a plot of c/m values versus atomic weight / molecular weight of anion / anionic salt shows a linear relationship (Figure 3.3) . Thus c/m is the molecular weight of the anion salt or the multiple of molecular weight of the anionic salt and a constant . This relationship may be useful to correlate the spot-area with the atomic weight of anions or molecular weight of anionic salts .

3.4 CONCLUSION

The chromatographic system consisting of cellouse thin layers and 0.1M HCl-acetone (1:9) is the most suitable solvent system for the identification , separation , and quantitation of several anions . The determination of IO_3^- with preliminary separation from IO_4^- is important , as iodate is reduced to iodide in alkaline medium .

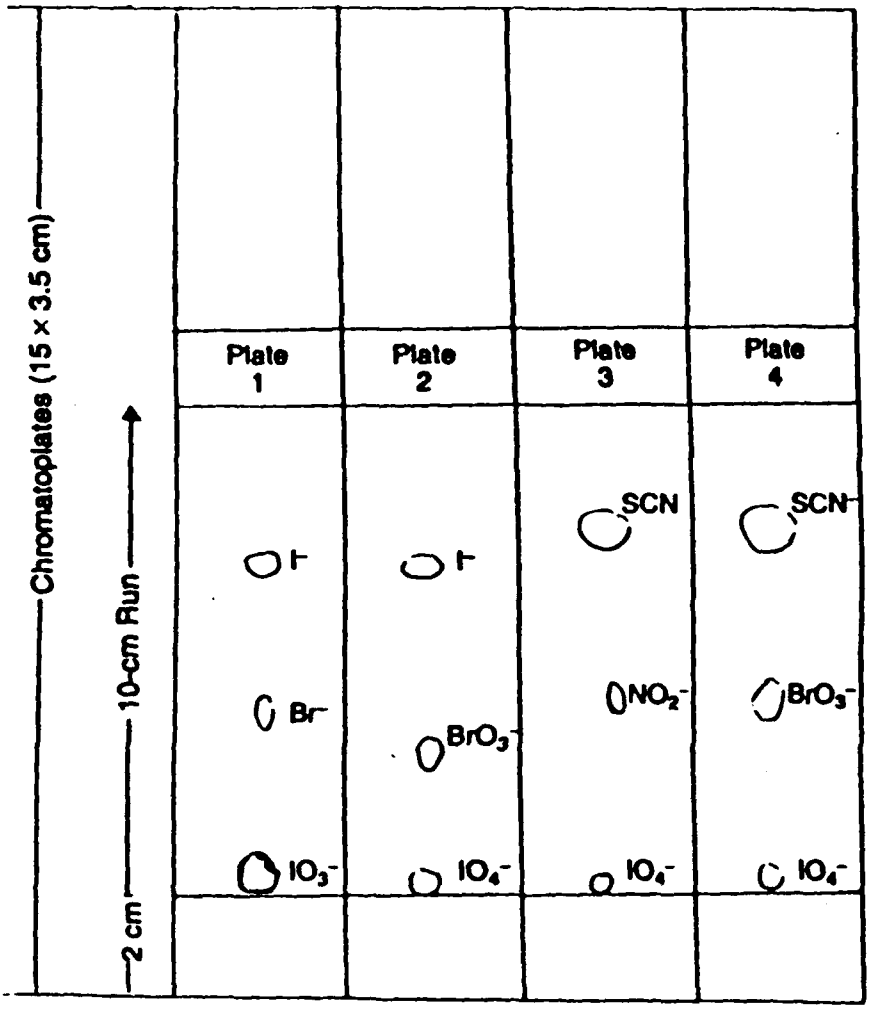


Figure 3.1 :Diagram of some ternary separations: 1, IO_3^- -BR^--I^-; 2, IO_4^- -BRO_3^- -I^-; 3, IO_4^- -NO_2^- -SCN^-; 4, IO_4^- -BrO_3^- -SCN^-. Conditions: stationary phase, cellulose; mobile phase, HCl–acetone (1:9).

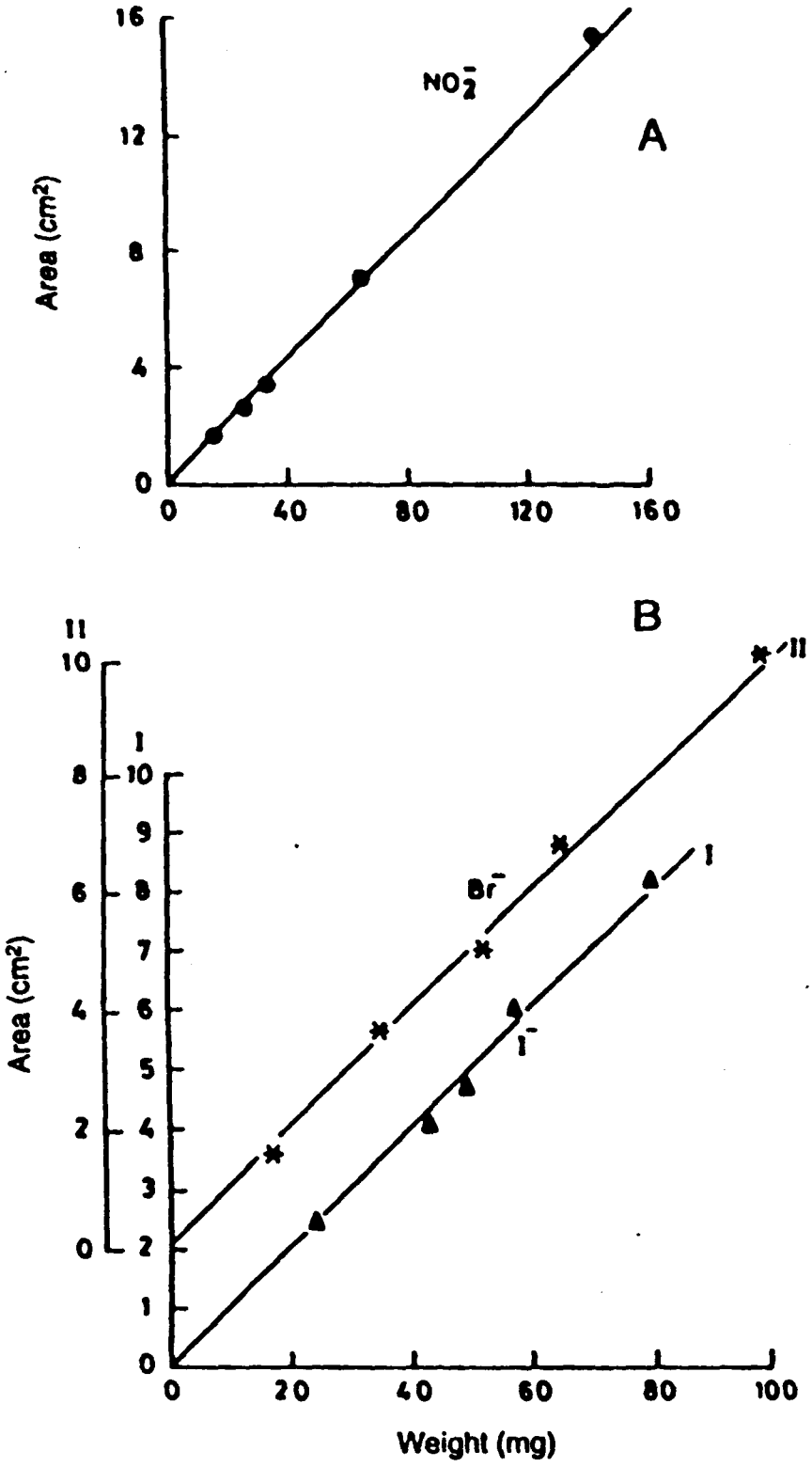


Figure 3.2 :Plot of spot area versus weight of paper covering the spot area. Conditions: stationary phase, silica gel impregnated with 1% $CuSO_4$; eluant, HCl-acetone (1:9).

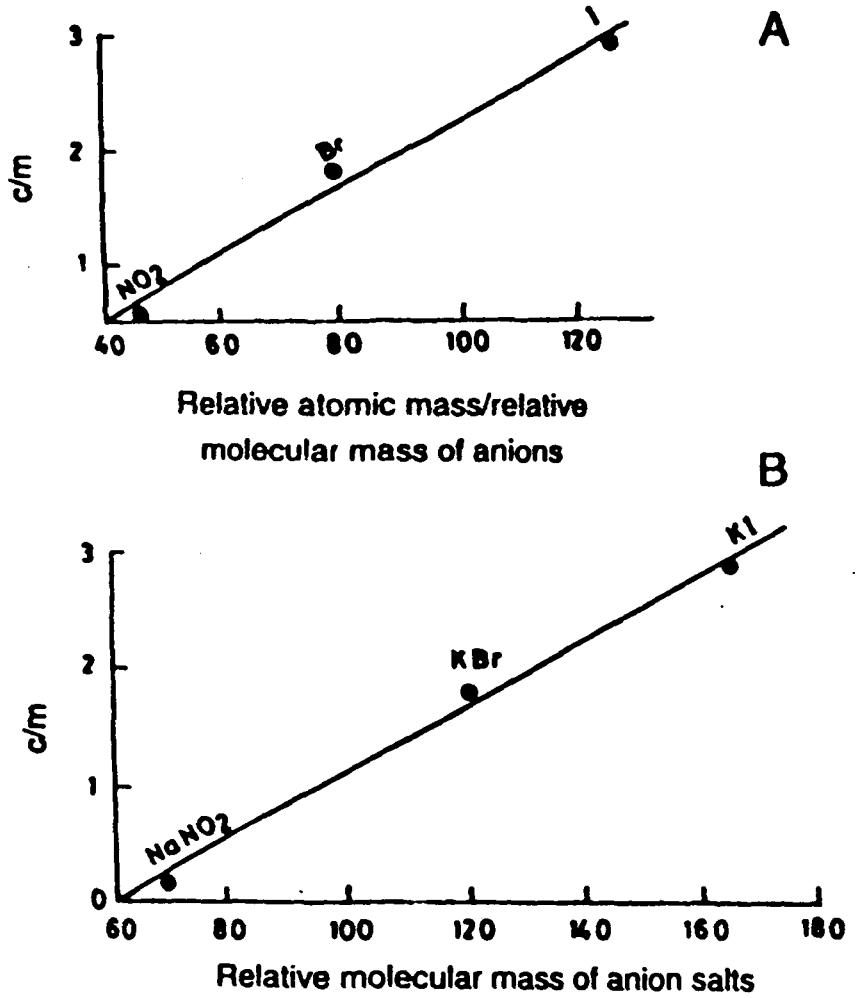


Figure 3.3: Plot of c/m values versus atomic weight/molecular weight of anion/anionic salt. Conditions: stationary phase, silica gel impregnated with 1% $CuSO_4$; eluant, HCl–acetone (1:9).

REFERENCES

1. J.G. Kirchner , in *Thin -Layer Chromatography : Quantitative , Environmental and Clinical Applications* , J.C. Touchstone and D. Rogers, (Eds.) Wiley Interscience ,New York (1980) .
2. E. Stahl , in *Thin -Layer Chromatography* , 2nd edn., E. Stahl (Ed) Springer -Verlag , Berlin , Germany (1969) .
3. A.Mohammad and K.G.Varshney,in *Handbook of Thin -Layer Chromatography* , J. Sherma and B.Fried (Eds.), Marcel Dekker ,New York (1990) .
4. R.Kuroda and M.P.Volynets, in *CRC Handbook of Chromatography: Inorganics* , Vol I, M. Qureshi (Ed.), CRC Press, Boca Raton. FL (1987).
5. A .Mohammad and M.A. Majid Khan, *J.Chem. Environ. Res.* 1(2) (1992) 3.
6. A .Mohammad and N. Fatima , *Chromatographia* 23 (1987)653 .
7. U.A.Th. Brinkman ,G. de Vries,and R.Kuroda, *J. Chromator.* 85(1973)187.
8. J.Sherma , *Anal. Chem.* 58(1986)69R .
9. J.Sherma, *Anal. Chem.* 60(1988)74R .
10. J.Sherma , *Anal. Chem.*64(1992)134R .
11. A.Mohammad and S.Tiwari, *Chromatographia* 30 (1990)405 .
12. A.Mohammad and S. Tiwari, *Microchem. J.*44(1991)39 .
13. A.Mohammad and S.Tiwari.*J.Planar Chromatogr.-Mod. TLC* 4 (1991)485.
14. W.W Scot, in *Standard Methods of Chemical Analysis*, 5th edn., Van Nostrand, New York (1993) .
15. A.Mohammad and S. Tiwari, *Microchem. J.*47(1993)379 .

CHAPTER -IV

W/O MICROEMULSION AS MOBILE PHASE IN THIN LAYER CHROMATOGRAPHIC RETENTION STUDIES OF ANIONS

4.1 INTRODUCTION

Microemulsions are isotropic, clear or translucent thermodynamically stable dispersions of oil , water , surfactant and often a small amphiphilic molecule, called a cosurfactant (1) . The droplet diameters in microemulsions range from 100 to 1000 Å .The microemulsion systems are usually transparent because of their much smaller droplet size compared to macroemulsions . One can distinguish three different types of microemulsions: Oil microdroplets enclosed in a surfactant-cosurfactant film are dispersed in an aqueous continuous phase (oil-in-water, O/W type); water droplets are dispersed in the oil-continuous phase (water-in-oil, W/O type); and if oil and water droplets overlap in each other , the system is called bicontinuous (2) . Water-in-oil (W/O) microemulsions are complex fluids that have wide-ranging applications; for example, as novel lubricants, reaction media for new synthetic chemistry and mobile phases in separation science (3,4) . Since the first report by Armstrong and Henry (5), micellar chromatography has been a subject of interest to many analytical chemists (6-8) . It has recently been shown that W/O microemulsions could be used as normal liquid chromatographic mobile phases (9). Interesting selectivities, alongwith poor efficiencies, were obtained. It must be noted that the W/O microemulsions used did not contain any alcohol cosurfactants. It was reported earlier that the addition of 1-pentanol to the mobile phase greatly improves efficiencies by reducing the adsorbed amount of emulsifier (surfactant). In this work , a W/O microemulsion with 1-pentanol as cosurfactant was used as eluant because use of such phases in thin-layer chromatography (TLC) is an unexplored area of research . In these systems,

water microdroplets , enclosed in surfactant/cosurfactant film, are dispersed in an oil-continuous phase (10) . One characteristic feature of microemulsions is their wide domain of existence in the pseudoternary phase diagram (9,11).

In continuation of our earlier studies on TLC of anions (12-14) , this report describes simple methodology for identification and separation of anions under optimal experimental conditions with a W/O microemulsion as novel eluant. The use of such phases may offer unique solutions for the change in retention data due to the influence of water on the stationary phase (15) because water is present in the core of W/O microemulsion droplets .

4.2 EXPERIMENTAL

***Apparatus*:** As reported in 3.2 .

***Chemicals*:** Specially pure sodium dodecyl sulfate (SDS) and n- heptane (99%) were obtained from BDH (Poole, United Kingdom) and n-pentanol was Riedel product (99%). Demineralized water, redistilled from alkaline potassium permanganate was used. Alumina, microcrystalline cellulose, and kieselguhr were obtained from CDH Laboratories (India) , while silica gel G was obtained from Glaxo (India) . All other chemicals (Analar grade) were used as supplied .

***Test Solutions*:** The test solutions (1%) were either Na or K salts of all anions studied , except SCN^- , which was used as ammonium thiocyanate. Solutions of the nitrates of lead, silver and bismuth , and the chloride of mercury were prepared in demineralized water , which contained small quantities of the corresponding acid to limit the extent of hydrolysis .The solutions (1%) of various amines and phenols used were prepared in methanol .

Detection Reagents : The spray reagents used for the detection of various anions were similar to those presented in 3.2 .

Mobile Phase : The W/O microemulsion, used as mobile phase, was prepared by titrating a coarse emulsion of n-heptane (160 ml), water (8 ml), and SDS (8 gm) with n-pentanol (24 ml) . Heptane was chosen for the oil phase because of low toxicity of the odd-carbon number alkanes . The microemulsion was produced at 30^0 C .

Stationary Phases: The following sorbent layers were used as stationary Phases ; Silica gel G (S_1) , Alumina (S_2), Microcrystalline cellulose (S_3), Kieselguhr G (S_4), Kieselguhr + cellulose (4:1,3:2) (S_5) .

Procedure

Preparation of TLC plates : The TLC plates were prepared by adopting the method as given in 3.2.

Chromatography : A sample volume (1.0 - 10 μL) with a sufficient amount of analyte (0.1-10 μ g) was applied with the aid of micropipette about 2.0 cm above the lower edge of the TLC plate . The spots were dried and the plates were developed by the one-dimensional ascending technique , in glass jars that contained the mobile phase . Before the development of plates, the jars, containing mobile phase , were covered with a lid for about 20 min, so that the jars may get pre-saturated with the mobile phase vapors. The solvent was allowed to migrate up to 10 cm from the starting line in all experiments. After development , plates were dried at room temperature, and the spots were visualized with the appropriate spraying reagent.

The identification limits of various anions were determined by spotting different amounts of anionic solutions on the chromatoplates. The plates

were developed and the spots were detected as described in 3.2 . The method was repeated with successive lowering of the amount of anion until spots could no longer be detected. The minimum amount of anion just detectable was taken as the limit of detection.

4.3 RESULTS AND DISCUSSION

We have tried identification and separation of anions on various adsorbents (S_1-S_5) .Kieselguhr thin layers were highly effective for differential migration of anions.Table 4.1 summarizes some binary separations of IO_4^- and MnO_4^- from accompanying anions by using the W/O microemulsion as mobile phase and kieselguhr as the stationary phase. Separations achieved are well resolved and compact in this chromatographic system . It is an important aspect of this study because our past experience (14) has shown that kieselguhr , being only slightly active, fails to provide any fruitful data when used as stationary phase for normal TLC of anions with organic or mixed aqueous-organic solvent systems. This indicates that the W/O microemulsion plays a crucial role in enhancing the differential migration of anions on kieselguhr.This is possibly due to the presence of a water core in W/O microemulsion droplets which provide a restricted volume (16) and are responsible for specific selectivities. The hR_F values of separated anions are given in Table 4.1 . The R_F value of each individual anion changes slightly when it is chromatographed in mixture with other anions .

Table 4.2 presents the detection and dilution limits of anions . The proposed method is highly sensitive for most ions, except Br^-,I^- and IO_4^-. The most interesting behavior is exhibited by MnO_4^- ,which can be detected easily , even if it is present at 1 μg. Similarly , CrO_4^{2-} and BrO_3^- can be

detected easily . This may well be attributed to the fact that , in W/O microemulsion , these solutes are localized in the hydrophilic core, which is responsible for some sort of preconcentration of anions (17) . Further , the anionic charge on the microemulsion droplet is responsible for fast transfer of anionic species out of the microemulsion droplet and easily available to interact with the detection reagents .

Table 4. 3 presents some quantitative separations of anions. KBr, up to 5 mg , and $NaNO_2$, up to 1.25 mg , can be easily separated from KIO_4 (100 μg). Separation of KIO_4 from milligram quantities of KI does not take place ,KIO_4 remains undetected while KI produces a tailed spot .Thus increasing the KI amount produces a deleterious effect and causes poor or no detection of KIO_4 .This may possibly be due to reduction of KIO_4 to iodine in solutions of moderate acidity. Because of this fact , we carried out the separation of IO_4^- from NO_2^- , Br^- , and I^- .

Finally, Table 4.4 summarizes the effect of various additives on the separation efficacy of IO_4^- from Br^- and NO_2^- . Amines by and large do not influence the separations, but there are drastic changes in the R_F values of ions,compared with their standard R_F values . The separation mixture precipitates by the addition of amines . In case of β-naphthylamine, Br^- remains undetected because the entire spot (R_F range of Br^-) is superimposed by the solvent uptake of β-naphthylamine (deep purple spot). The situation for NO_2^- is similar,but the spot of NO_2^- is clear. Spots of Br^-, when chromatographed with diphenylamine and 2-nitroaniline , appeared as streaks after $^1/_2$ h. Phenols neither hamper separation nor produce precipitation . However, addition of pyrogallol produces slight turbidity, and IO_4^- remains

undetected when separated from NO_2^- or Br^-. In this case, the R_F range of IO_4^- gets superimposed by pyrogallol. Heavy metals also do not hinder the separation except silver, which causes dense precipitation as a result of which Br^- remains undetected .Addition of lead to the separation mixture of IO_4^- and Br^-, leads to poor detection of Br^-. Mercury shows no effect on the separation of IO_4^- from Br^- or NO_2^-.

An attempt has been made for the semi-quantitative determination of IO_4^- by employing a peak-height measurement procedure . For this purpose, 0.1 ml standard aqueous solution of KIO_4 (0.02 - 0.1, %) were spotted on kieselguhr thin layers. The chromatograms were developed and detected. The spots obtained were copied directly on tracing paper from the chromatoplates . A linear relationship was obtained when the square of peak -heights of the spots, was plotted against microgram quantities of KIO_4 (Figure 4.1) .

Table 4.1 : Binary Separations Achieved Experimentally on Kieselguhr Layers Developed with W/O Microemulsion

Stationary phase	Separations ($hR_F = R_F \times 100$)
Kieselguhr G	IO_4^- (5.75) – NO_2^- (89.5)
	IO_4^- (6.75) – BrO_3^- (89.25)
	IO_4^- (7.25) – I^- (88.25)
	IO_4^- (7.5) – Br^- (94.0)
	MnO_4^- (5.0) – BrO_3^- (91.0)
	MnO_4^- (5.0) – NO_2^- (90.0)
	MnO_4^- (2.5) – Br^- (93.25)

Table 4.2 : Detection and Dilution Limits of Anions as Their Salts on Kieselguhr Layers Developed with W / O Microemulsion

Anion	Lower detection limit (μg)	Dilution limit
IO_4^-	20	1:1000
IO_3^-	10	1:2000
BrO_3^-	2	1:10,000
I^-	20	1:1000
Br^-	20	1:1000
MnO_4^-	1	1:20,000
CrO_4^{2-}	2	1:10,000

Dilution limit = 1: (Volume of test solution× 10^6 [Limit of detection (μg)]

Table 4.3 : Quantitative Separation of IO_4^- (20– 100μg) from Milligram Amounts of NO_2^-, Br^-, and I^- with Chromatographic System as in Table 4.1

Accompanying anion salt	Amount of salt (mg)	Separations ($R_L - R_T$)
$NaNO_2$	1.25	IO_4^- (0.15–0.00) – NO_2^-(1.0-0.46)
KBr [a]	1.25	IO_4^- (0.11–0.00) – Br^-(1.0-0.76)
	2.5	IO_4^- (0.10–0.00) – Br^-(1.0- 0.68)
	5.0	IO_4^- (0.10–0.00) – Br^-(1.0–0.48)
KI	1.0	IO_4^- (ND[b]) – I^-(1.0–0.70)
	2.0	IO_4^- (ND) – I^- (1.0–0.65)
	4.0	IO_4^- (ND) – I^- (1.0–0.20)

[a]Spots of Br^- appear after 20 min ; [b] ND = Not detected

Table 4.4 : Effect of Additives on Some Binary Separations of IO_4^- from NO_2^- and Br^-

Additives	Separations(R_F)
1. Amines	
(a) β –Naphthylamine	IO_4^- (0.05) – NO_2^- (0.85) ; IO_4^- (0.06) – Br^- (ND)
(b) Diphenylamine	IO_4^- (0.21) – NO_2^- (0.88) ; IO_4^- (0.21) – Br^-(0.74)
(c) 2– Nitroaniline	IO_4^- (0.20) – NO_2^- (0.65) ; IO_4^- (0.17) – Br^-(0.78)
2. Phenols	
(a) Phenol	IO_4^- (0.03) – NO_2^- (0.82) ; IO_4^- (0.10) – Br^-(0.85)
(b) Resorcinol	IO_4^- (0.07) – NO_2^- (0.90); IO_4^- (0.07) – Br^- (0.81)
(c) Pyrogallol	IO_4^- (ND) – NO_2^- (0.80) ; IO_4^- (ND) – Br^- (0.81)
3. Heavy metals	
(a) Hg^{2+}	IO_4^- (0.05) – NO_2^- (0.92) ; IO_4^- (0.08) – Br^-(0.83)
(b) Pb^{2+}	IO_4^- (0.03) – NO_2^- (0.90) ; IO_4^- (0.01) – Br^- (ND)
(c) Ag^+	IO_4^- (0.05) – NO_2^- (0.94) ; IO_4^- (0.05) – Br^- (ND)
(d) Bi^{3+}	IO_4^- (0.04) – NO_2^- (0.94) ; IO_4^- (0.06) – Br^- (ND)

Standard R_F values of individual ions are: IO_4^-(0.00), NO_2^-(0.92), and Br^-(0.90).

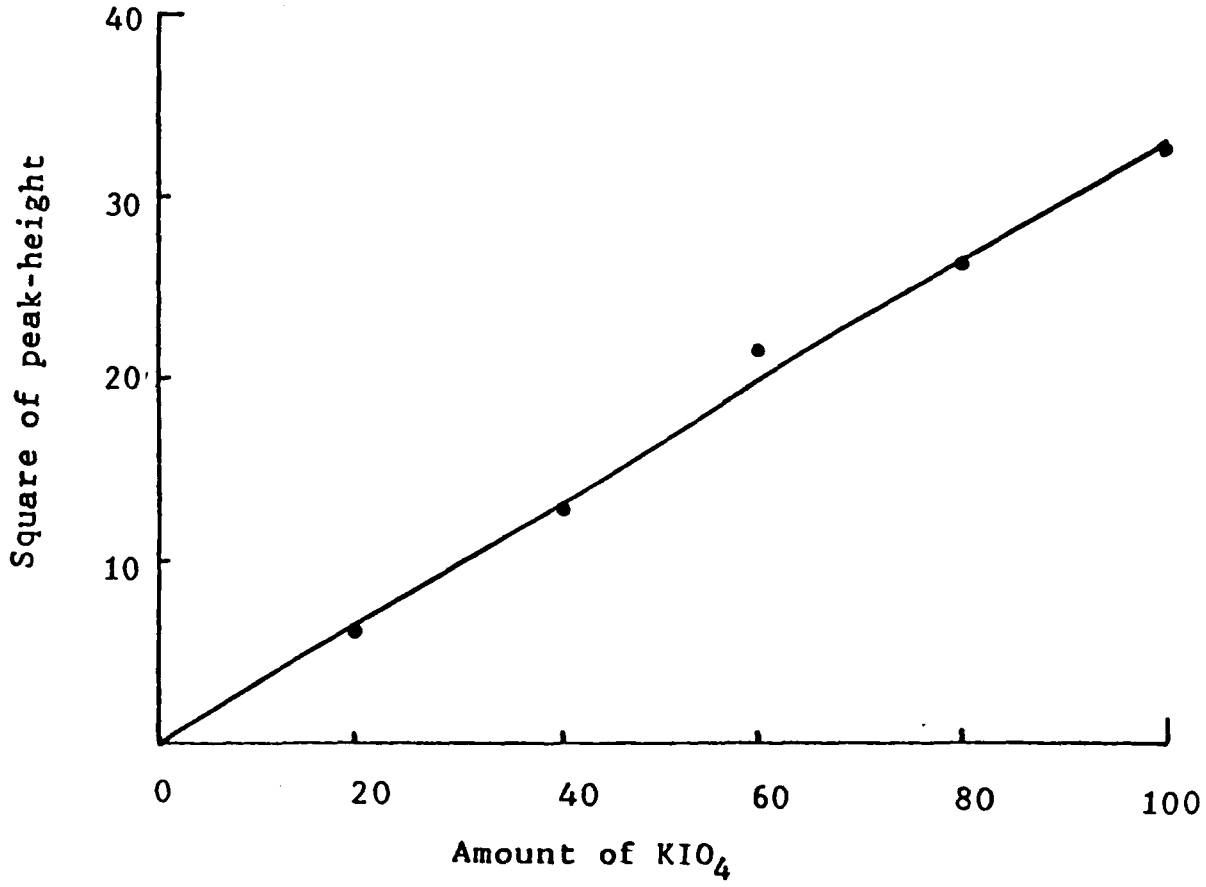


Figure 4.1: Standard calibration curve for semiquantitative determination of IO_4^- by peak-height measurement method.

REFERENCES

1. K. Shinoda and S.Frieberg, *Adv. Colloid interface Sci.* 4 (1975) 241.

2. L.M. Prince, in *Microemulsions: Theory and practice,* Academic Press, New York(1977).

3. B.H. Robinson, *Chem. Br.* 26(1990)342.

4. A.Berthod and M.D. Carvalho, Anal . Chem . 64 (1992) 2267 .

5. D.W. Armstrong and S.J. Henry , *J.Liq. Chromatogr.* 3(1980) 657.

6. L.J.Clive Love, J.G. Habarta and J.G. Dorsey , *Anal. Chem* .56(1984) 1132A .

7. M.A.Hernandez-Torres, J.S. Landy and J.G.Dorsey, *Ibid.* 58 (1986) 744 .

8. D.W.Armstrong, and W.L.Hinze, *Use of Orderd Media in Chemical Separations,* ACS Symp. series 342 ,American Cemical Society, Washington D.C.(1987).

9. A.Berthod,O.Nicolas and M. Porthault, *Anal.Chem.* 62(1990) 1420 .

10. S.Kumar and H.N. Singh, *Colloids Surfaces* 44(1990) 17 .

11. S. Kumar and H.N. Singh, *J.Surf. Sci. Technol.* 5(1989) 60 .

12. A.Mohammad, and S. Tiwari , *Microchem.J.* 44(1991) 39.

13. A. Mohammad and S. Tiwari , *Microchem.J.* 47(1993) 379 .

14. A.Mohammad, S. Tiwari and J.P.S.Chahar,*J.Chromatogr. Sci.* 33 (1995) 143.

15. C.Souteyrand, M.Thibert, M.Caude and R. J.Rosset,*J.Chromatogr* . 316(1984) 373.

16. J.H.Fendler, *Chem. Rev.* 87(1987) 877 .

17. J.H. Fendler, *Acc . Chem. Rev.* 9 (1976)153 .

CHAPTER -V

THIN LAYER CHROMATOGRAPHIC SEPARATION, COLORIMETRIC DETERMINATION AND RECOVERY OF THIOCYANATE FROM PHOTOGENIC WASTE, RIVER AND SEA WATERS

5.1 INTRODUCTION

Identification , separation and quantification of SCN^- is important due to its application in photography , catalysis , agri- chemicals , rust - inhibition and dyeing - printing of textiles(1). At high acidities , thiocyanate produces cyanides in the presence of oxidants and thus causes lethal damage to aquatic life when SCN^- containing waste is discharged into rivers . Numerous methods (2-8) have been developed for analysing SCN^- and CN^- but most of them were not applied to polluted water and effluents . A spectrophotometric method based on the color reaction of Fe^{3+} with SCN^- has been used for the quantification of SCN^- but it suffers from cationic , anionic and phenolic interferences . An extensive literature survey on the quantitative determination of SCN^- (1981-1992) with respect to the techniques used , shows that out of twenty research papers published , only one dealt with spectrophotometry .

TLC has been a widely used technique for the separation of SCN^-(9-12) . The literature survey(13) shows that out of forty research papers published so far on TLC of SCN^- , none examined the separation of SCN^- from complexing cations (Ag^+, Fe^{3+} , Co^{2+},Cu^{2+}, etc.). This chapter reports a reliable TLC- colorimetric method for the determination of SCN^- in the presence of cationic and anionic impurities . The proposed method is applicable to the determination of SCN^- in photogenic waste , river water and seawater .

5.2 EXPERIMENTAL

***Apparatus*:** TLC applicator, glass plates and jars were as repoted in 3.2. Spectrophotometer (Elico,India, Model Ultra Spec. CL-54) and pH meter

(Elico , India , Model LI - 10T) were used .

Chemicals **:** Ammonium thiocyanate (Merck , India); ferric chloride and ammonia solution (Qualigens); acetone , cellulose microcrystalline and Kieselguhr G (CDH , India) were used . All other chemicals were of Analytical Reagent grade .

Test Solutions **:** Aqueous solutions (1.0% w/v) of following were used .

(a) Salts of anions were as reported in 3.2

(b) Nitrates of Ag^{+} ,Bi^{3+}, UO_2^{2+} and Zn^{2+}

(c) Chlorides of Fe^{3+} and Hg^{2+}

(d) Sulfates of Cu^{2+}, VO^{2+}, Co^{2+} and Mn^{2+}

Detection Reagents **:** To locate the spots of analyte ions, the reagents used were :

(a) For anions detection reagents were as mentioned in 3.2 .

(b) 1.0% $K_4Fe(CN)_6$ in water for Fe^{3+} , Cu^{2+} , VO^{2+} and UO_2^{2+} .

(c) 1.0% Dimethylglyoxime in ethanol for Co^{2+}.

(d) 1.0% Dithizone in benzene for Zn^{2+} , and Cd^{2+} .

(e) Saturated H_2S in water for Ag^{+} , Hg^{2+} and Bi^{3+} .

Mobile Phases **:** Mixtures of 0.1 M NH_4OH and CH_3COCH_3 in 1:9 (M_1), 3:7 (M_2) , 1:1 (M_3), 7:3 (M_4) and 9:1 (M_5) ratios were used as solvent systems .

Stationary Phases**:** Plain cellulose microcrystalline (S_1), Kieselguhr G (S_5) and their mixture in 4:1 (S_2), 3:2 (S_3) and 1:1 (S_4) ratios were used as adsorbent meterials .

Procedure**:** The entire methodology was carried out as follows :

Preparation of TLC plates :Plates were prepared as mentioned in 3.2 .

Preparation of fortified samples : Four samples of SCN^- solutions (5.09-12.92 mM) were prepared in seawater (collected from Anjuna Beach ,Goa, India). Similarly , spiked river water (Ganga , Rajghat) and photogenic waste (Aligarh) were prepared to examine the recovery of SCN^-.

Chromatography of ions : To study the retention behavior of some inorganic ions , 0.01 ml of the test solution was spotted onto the TLC plate, air dried and then developed with M_1- M_5 upto 10 cm. The plate was air dried, analyte ions were detected as colored spots and then identified on the basis of their R_F values . For mutual separations , an aliquot (0.02 ml) of binary or 0.03 ml of ternary mixture of analytes was loaded onto the plate and the chromatography was performed . In order to examine the effect of sample pH on the separation of SCN^- ,various mixtures of test solutions were brought to the required pH value (3.0-12.25) by adding either glacial acetic acid or NaOH solution . The effect of amines (2-nitroaniline, ß-naphthylamine and diphenylamine) and phenols (phenol, resorcinol and pyrogallol) were examined on the detection and separation of SCN^- by mixing 1% alcoholic solution of additive with binary / ternary mixture containing SCN^-.

Colorimetry of thiocyanate : For quantitative determination of SCN^- by TLC - colorimetry , 0.1 ml of standard ammonium thiocyanate (2.55 -25.48 mM SCN^-) was spotted onto the plate (S_2) alongwith foreign ions and developed with M_1 . A pilot plate was simultaneously run . SCN^- containing portion of adsorbent from the working plate was scraped off and SCN^- was extracted with 10 ml of distilled water followed by washing of the adsorbent with 2 ml of water. Thus the total volume of solution was kept to 12 ml in each case . To the filtrate, 0.5 ml of chromogenic reagent solution

(8.0 % ferric chloride in 2 M HCl) was added. Absorbance was measured at 460 nm and the absorbance was plotted against the concentration (ppm) of SCN^- solution to sketch the standard calibration curve which was used to determine the concentration of SCN^- in a water sample with preliminary separation from accompanying inorganic ions.

Recovery from fortified samples : For the recovery of SCN^- from spiked waste (photogenic) and water (river and sea) samples, 0.1 ml of the solution was spotted onto TLC plate (chromatographic system ; S_2, M_1) and the chromato-colorimetry was performed in the similar manner as that for standards. The loaded and recovered amounts of SCN^- were compared and the relative standard deviation (%) , relative error and percent recovery were calculated .

5.3 RESULTS AND DISCUSSION

Qualitative Studies **:** Inorganic ions were chromatographed on plain and mixed adsorbents containing cellulose and /or Kieselguhr (S_1- S_5) using mixtures of 0.1 M NH_4OH and CH_3COCH_3 in various ratios as mobile phases (M_1-M_5) . The results of this study are summarized in Tables 5.1and 5.2 . The effect of proportional composition of mobile phase on the hR_F values of inorganic anions is examined and the results are reported in Table 5.1 where only plain adsobents ($S_1$and S_5) were used . Cations were also chromatographed with these chromatographic systems, cations remain at the point of application . However, some metal ions yield occasional tailed spots. To achieve difference in migration of anions, $S_1$and S_5 were mixed together to get a set of adsorbents (S_2-S_4) on which the anions were chromatographed using M_1as mobile phase. The hR_F values of anions are

enlisted in Table 5.2 .Out of three mixed adsorbents, cellulose -Kieselguhr (4:1,S_2) is selected because it provides sufficient difference in hR_F values of SCN^- and others. It also provides compact spots because increasing Kieselguhr proportion results in the tailing of spots. Hence, the combination of S_2 and M_1 is the best chromatographic system which is utilized for the selective separation of SCN^- from all ions studied . These ions strongly interfere in the colorimetric method of SCN^- quantification due to the formation of colored complexes either with SCN^- or Fe^{3+} (14). For Ag^+-SCN^- separation, the white precipitate of AgSCN formed on mixing of both ions was dissolved in NH_3 solution and the clear solution was spotted on the TLC plate. Fe^{3+} -SCN^- separation is of great importance, especially when SCN^- is to be determined colorimetrically by using $FeCl_3$ as chromogenic reagent .

Fot this separation, 1.0 % aqueous NaF solution was added to red colored complex to decolorize it . FeF_3, a more stable complex was formed and SCN^- became free and was detected on TLC plate .In the Co^{2+} -SCN^- separation, Co^{2+} could not be detected . In general, for metal ion -SCN^- separation, the amount of SCN^- was taken x times more than that of metal ion (x is the number of unit charges present on the metal ion). Effects of sample pH, phenols and amines on the detection of SCN^- have been examined and SCN^- was clearly detected in all cases. The R_F value of SCN^- remained unchanged over the pH range (3.0-12.25) of sample solution . No change in the mobility of SCN^- was noticed on being chromatographed in the presence of amines and phenols .

Quantitative Studies : The proposed TLC system (S_2 ,M_1) was coupled to the spectrophotometry of SCN^- using Fe^{3+}- SCN^- color reaction in an acidic (2 M HCl) medium. The complex absorbs incident radiation of 460 nm and follows the Beer's law up to 11.18 ppm .The optical density (A) plotted against the concentration (ppm) of SCN^- is an average of A_1 (SCN^-after separation from NO_2^-) , A_2 (from CrO_4^{2-}) and A_3 [from $Fe(CN)_6^{3-}$] . The R.S.D. varies from 2.124 -0.442 % .

To test the applicability, proposed TLC- colorimetric method was applied to the fortified samples of photogenic waste, river water and seawater. The results are summarized in Table 5.3 . This table shows that the relative recoveries of SCN^- are almost the same .

Table 5.1 : Effect of Mobile Phase Composition on the Mobility of Inorganic Ions[a]

Anion	Stationary phase	hR_F values achieved with				
		M_1	M_2	M_3	M_4	M_5
SCN^-	S_1	49	91	91	93	95
	S_5	85	90	90	90	92
NO_2^-	S_1	21	76	82	91	93
	S_5	88	89	90	90	93
MnO_4^-	S_1	0	0	0	0	0
	S_5	0	0	0	0	0
$Fe(CN)_6^{3-}$	S_1	6	54	89	92	95
	S_5	83	90	90	92	93
$Fe(CN)_6^{4-}$	S_1	0	6	78	90	96
	S_5	8	17	80	91	93
CrO_4^{2-}	S_1	10	23	70	90	95
	S_5	41	91	91	93	94
$Cr_2O_7^{2-}$	S_1	8	20	78	88	92
	S_5	43	89	90	90	94
MoO_4^{2-}	S_1	9	24	83	90	93
	S_5	36	88	90	91	96
$Mo_7O_{24}^{6-}$	S_1	12	30	80	92	92
	S_5	38	90	91	95	95

[a]*Metal ions remain at the point of application in all of the above mentioned chromatographic systems.*

Table 5.2 : Effect of Stationary Phase Composition (Cellulose / Kieselguhr Proportion) on the Mobility of Inorganic Ions* when Developed with M_1

Anion Studied	hR_F values achieved on				
	S_1	S_2	S_3	S_4	S_5
SCN^-	49	79	80	85	85
NO_2^-	21	21	32	86	88
MnO_4^-	0	0	0	0	0
$Fe(CN)_6^{3-}$	6	6	7	12	83
$Fe(CN)_6^{4-}$	0	0	0	0	0
CrO_4^{2-}	10	20	24	28	41**
$Cr_2O_7^{2-}$	8	19	19	25	43
MoO_4^{2-}	9	11	17	21	36**
$Mo_7O_{24}^{6-}$	12	15	15	23	38

*All metal ions studied remain at the point of application (hR_F =0.0).

**Tailed spots (hR_L- hR_T > 30) .

Table 5.3: Recovery of SCN^- from Fortified Environmental Samples

Sample (pH)	Amount of SCN^- loaded (μg)	Amount of SCN^- recovered (μg)	Relative recovery (%)	Relative standard deviation (%)
Photogenic waste	29.60	30.19	102.00	9.38
(9.4)	44.40	45.18	102.44	5.60
	59.20	60.64	102.44	5.42
	74.00	76.46	103.32	10.70
River water	29.60	28.12	94.99	9.91
(8.2)	44.40	42.62	96.00	7.84
	59.20	57.22	96.66	7.09
	74.00	73.03	98.69	5.63
Sea water	29.60	27.38	92.50	9.91
(8.0)	44.40	41.64	93.78	7.57
	59.20	56.23	94.99	6.59
	74.00	71.53	96.66	5.45

REFERENCES

1. C.T. Nicolas and A.J. Bernard , *Anal.Chem.* 55(11) (1983)1667 .
2. W.J. Williams, in *Handbook of Anion Determination* ,Butterworth , London(1979) 228.
3. L.Zhihong, T. Shizong and C. Jieke, *Sepu* 7(1989) 44.
4. A. Mohammad and S.Tiwari, *Chromatographia*, 30(1990) 405 .
5. T.Shinzo, T.Toshihiko, O.Koreharu,T. Kiyomi and I.Toshio, *Bunseki Kagaku* 30 (1981) 30 .
6. T. Akira, D.Kazuhiro and D. Toshio, *Anal. Chim. Acta.* 261 (1992) 281 .
7. C.S. Hwei , W.H. Lung, T. Minoru , S. Toshiyuki and F. Koichi, *Chem. Abstr.* 113(1990) 17112 n .
8. I. Toshio, T. Shinzo, T.Toshihiko and T. Kawanishi, *J. Chromatogr.* 250(1992) 55 .
9. J. Franc and E. Kosikova, *J.Chromatogr.* 187 (1980) 462 .
10. U.A.Th. Brinkman, G. De Vries and R.Kuroda , *J.Chromatogr.* 85 (1973) 187 .
11. A. Mohammad and K.G. Varshney , in *Handbook of Thin-Layer Chro matography*,55, J.Sherma and B. fried (Eds.), Marcel Dekker, New York (1990) 463 .
12. J.Sherma , *Anal. Chem.* 64 (1992) 134R .
13. A.Mohammad and S. Tiwari , *Sep. Sci . Technol.* 30 (19) (1995) 3577.
14. G.Svehla , *Vogel's Quantitative Inorganic Analysis*,6th edn., Orient Longman , New Delhi (1987) 167 .

CHAPTER -VI

A NEW CHROMATO-IODOMETRIC METHOD FOR THE SEPARATION AND DETERMINATION OF IODIDE AND ITS OXYANIONS

6.1 INTRODUCTION

The determination of anions has undergone substantial changes in recent years . Significant advances have been made in the area of chromatographic separation and determination of inorganic species (1-4) . However, there is a need of rapid clean-up , identification and quantitation of inorganic anions present in environmental samples . Thin-layer chromatography (TLC) being inexpensive and simple, can be utilized as a reasonably rapid analytical tool to compliment modern instrumental techniques .

The literature survey of last thirty-five years on TLC of inorganics reveals that out of 148 papers published on inorganic anions (5) , 62 papers report the TLC of one or two anion(s) of iodine . There were only 19 papers which described the TLC of all three iodine anions (iodide, iodate and periodate). As regards to ternary separation of these anions under optimized experimental conditions , few workers have used silica gel (6-8), alumina (9,10) and mixed alumina (11) layers . All the papers published on TLC of iodide and its oxyanions describe only their qualitative studies , not their quantitative determination . Furthermore , none of these workers have examined the effect of transtion metal ions on the separation of iodine anions. The work reported here is aimed at solving the problem of determining iodide , iodate and periodate after their rapid clean-up on alumina 'G' layer using NH_4OH - CH_3COCH_3 mixture as mobile phase .

A new iodometric method is developed for simultaneous determination of I^-,$IO_3^-$and IO_4^- .This method requires lesser reagents because analytes are used as iodine producing agents for one another and the CH_3COCH_3 is utilized as titrant as well as a component of mobile phase . The proposed

method is applicable to the spiked double distilled water for the recovery of analytes.

6.2 EXPERIMENTAL

Apparatus :TLC Applicator, plates and jars used were as given in 3.2.

Chemicals : The chemicals used were KIO_3 , KIO_4 and CH_3COCH_3 (CDH, India) ;HNO_3 and NH_3 solution (Qualigens). All other chemicals used were of analytical reagent grade .

Test Solutions : The test solutions were 1.0%(except IO_4^- which was taken as 0.5%) aqueous solutions of potassium salts of iodide, iodate and periodate.

Detection Reagents :Following chromogenic reagents were used to locate the anionic spots on TLC plate ;

(a) 0.3% $(C_6H_5)_2NH$ in 4 M H_2SO_4 for IO_3^- and IO_4^-

(b) Saturated $AgNO_3$ in CH_3OH for I^-

Mobile Phases : Mixtures of 0.1M NH_4OH and CH_3COCH_3 in 1:9 (M_1) ,3:7 (M_2) ,1:1(M_3) , 7:3 (M_4) and 9:1 (M_5) ratios were used .

Stationary Phases : Plain adsorbents i.e. aluminium oxide 'G' (S_1) , cellulose microcrystalline (S_2) , kieselguhr 'G' (S_3) and silica gel 'G' (S_4) were used . All adsorbents were supplied by CDH (India).

Procedure :The complete methodology involves following steps ;

Preparation of TLC plates : TLC plates were prepared by adopting the method as given in 3.2 .

Identification of analytes : 0.01 ml of test solution was spotted onto TLC plate, spot was air dried and then developed by ascending mode fixing ascent upto 10 cm. After development, plate was dried again and then the anions were visualized as colored spots by spraying with appropriate

detection reagent . The analyte species were identified on the basis of their R_F values , calculated from R_L (R_F of leading front) and R_T (R_F of trailing front) for each spot .

Separation : The test solutions of iodide , iodate and periodate were mixed in proper ratio , the resultant mixture was spotted onto TLC plate coated with alumina and the chromatography was performed using M_5 as mobile phase . To examine the pH and time effects on the resolution of all three components from their mixtures , stock solutions of mixture were prepared by mixing KI , KIO_3 and KIO_4 solutions in 5:1:1 ratio and their pH was adjusted to a definite level (pH range 4 -10) by adding AcOH or NaOH solutions . An aliquot (0.1 ml) of each mixture was spotted on TLC plate at different time intervals . The plates were developed and the resolved spots were detected . For investigating the effect of transition metal ions on the separation of I^- , IO_3^- and IO_4^- , 1.0%aqueous solution of metal ion was mixed with ternary mixture of analytes to get a solution consisting of metal ion , iodide,iodate and Periodate in 2:5:1:1, ratio by volume . Of this solution, 0.1 ml was spotted onto TLC plate .In the case of precipitation , filtrate (0.1 ml) was spotted . After separation , analyte species were detected .

Quantitative determination : 0.1 ml of standard solution of KI (0.1 - 1.0%) , KIO_3 (0.02- 0.20%) or KIO_4 (0.02 -0.20%) was spotted separately onto the alumina coated plate (working plate) and the plate was developed with M_5 . A pilot plate was simultaneously run to locate the exact position of analyte spot . After development , TLC plate was completely dried at 60°C to make it free from traces of the developer and the area of adsorbent corresponding to analyte spot was scraped off and taken into a

conical flask followed by the addition of 10 ml of the following solution ;

(a) 0.05% (w/v) KIO_3 in 0.1M HNO_3 for I^- .

(b) 0.05% (w/v) KI in 0.1M HNO_3 for IO_3^- and IO_4^- .

Brown color was produced which was titrated directly (without filtration) with 2.0 M aqueous CH_3COCH_3 (v/v) until complete decolorization occurs .The volume of titrant so consumed was plotted against the amount of analyte substance .

Recovery from fortified water samples : The solutions of KI (0.2-0.6%) KIO_3 (0.04-0.12%) and KIO_4 (0.04-0.12%) were prepared in double distilled water from stock solutions of KI (10%), KIO_3 (1%) and KIO_4 (0.5%) .Three test samples were prepared by mixing equal volumes of KI , KIO_3 and KIO_4 solutions of the following concentrations .

(a) KI (0.2%) , KIO_3 (0.04%) and KIO_4 (0.04%)

(b) KI (0.4%) , KIO_3 (0.08%) and KIO_4 (0.08%)

(c) KI (0.6%) ,KIO_3 (0.12%) and KIO_4 (0.12%)

0.1 ml of one of above mixtures was spotted onto the TLC plate , chromatography was performed and the analytes were titrimetrically determined after mutual separation .The chromatography and titrimetry were performed in similar manner to that for standards , Percent recovery and relative standard deviation were calculated .

6.3 RESULTS AND DISCUSSION

Qualitative Studies : In order to find out the most suitable stationary phase for the separation of iodide , iodate and periodate , commonly used adsorbents i.e. silica gel, alumina ,cellulose and kieselguhr were tried .Out of these , the satisfactory separation was achieved only on alumina layer when deve-

loped with mobil phases (M_2 to M_5) , giving best separation with M_5 (Table 6.1) . Considering the resolution of detected spots , the chromatographic system comprising of S_1 and M_5 as stationary and mobile phases was selected for detailed study .

Alumina exhibiting both cation and anion exchange characteristics has many desirable properties such as rigid structure , little swelling / shrinking in water or solutions containing electrolyte and organic modifiers , reasonable resistance to strong oxidizing and reducing agents and good thermal stability . Its low resistance towards strong acids and bases make it useful for applications under mild acidic or basic conditions . It is assumed that in our case , the retention of analyte anion on alumina follows anion exchange as shown below :

$$Al^+ OH^- + X^- \rightleftharpoons Al^+ X^- + OH^-$$

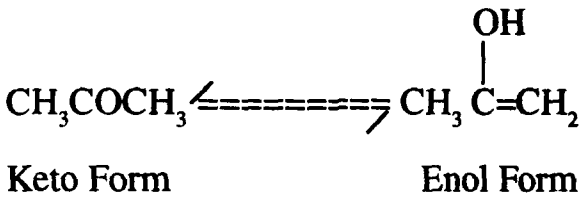
The separation of iodide , iodate and periodate is of great importance as these anions are interconvertible . Hence , this separation is selected for detailed qualitative as well as quantitative studies . It was always possible to detect iodide and iodate at all pH values (pH 4 - 10) in a three component mixture whereas periodate could not be detected when a sample (mixture of iodide , iodate and periodate) of pH 4.0 was chromatographed after 30 minutes of mixing time . Thus , all three components (iodide , iodate and periodate) can be resolved , detected and quantified on TLC plates from a single sample upto 30,60 and 75 min at pH values of 3, 6 - 7 and 10 respectively Fig. 6.1 .It seems that the reduction of IO_4^- into IO_3^- is slowed down in alkaline medium.Effect of some trasition metal ions (VO^{2+},Mn^{2+},Fe^{3+},Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}) which are encountered in a variety of cases, examined

on the separation ,IO_4^- - IO_3^- - I^-. The mixture consisting of KI , KIO_3 and KIO_4 in 5:1:1 ratio (pH =7) was chromatographed with and without metal ions using S_1/ M_5 chromatographic system . These metal ions remained at the point of application (R_F =0) when chromatographed individually with the same system . All three anions were resolved on TLC plate in the absence of metal ions . The addition of metal ions to the mixture leads to the formation of little precipitation at the bottom of glass tube in each case . Chromatography of supernatent shows the presence of metal ions alongwith well resolved spots of iodide and iodate . However , no clear detection of periodate was observed .The precipitate was dissolved in minimum amount of an acid (Fe^{3+},Co^{2+}) or NH_4OH (Cu^{2+} , Zn^{2+} and Ni^{2+}) and the TLC of this solution shows the presence of metal ions in the form of well formed spots.

Though , periodate was clearly detected on TLC plate after chromatography of dissolved precipitate produced in the case of Fe^{3+} , Cu^{2+} , Zn^{2+} , Ni^{2+} and Co^{2+} but it could not be detected in the case of VO^{2+} and Mn^{2+} . It seems that VO^{2+} and Mn^{2+} being reducing agents , convert periodate into iodate .This assumption finds support from the observation that the mixture of periodate and metal ion when chromatographed and detected , a spot corresponding to the R_F value of iodate was observed on TLC plate and periodate was found absent , It clearly indicates the transformation of periodate into iodate . VO^{2+} and Mn^{2+} were clearly detected at their original position (R_F = 0) .

Quantitative Studies : I^- (0.1-1.0 mg as KI), IO_3^- (20-200 µg as KIO_3) and IO_4^- (20-200 µg as KIO_4) have been quantitatively determined

titrimetrically using 2.0% aqueous CH_3COCH_3 as titrant at room temperature (20 ± 2°C). The analyte solutions produce yellow/brown color of iodine on addition of appropriate reagent . The addition of acetone to the solution results in the decolorization due to the iodination of acetone . The equivalence point is reached when brown /yellow color is changed to colorless . Volumes of titrant so consumed are plotted against the amounts of analyte salts . It is expected that experimentally more volume of acetone be consumed for each mole of analyte as required theoretically on the basis of reaction mechanism . In the presence of an acid or base an equilibrium between keto and enol forms of acetone exists as a result of direct shift of hydrogen from carbon to oxygen and vice-versa ,as ;



The keto form is more stable and the percentange of enol content of acetone is 1.5×10^{-4} (12) . The extent of enolization is affected by solvent, concentration and the temperature . Water is capable to reduce the enol concentration by hydrogen bonding with carbonyle group . In the present case , enolic form is solely responsible for iodination and therefore the consumption of acetone taken as 2.0 % aqueous solution is much larger than the theoretical value . The standard calibration curves for KI ,KIO_3 and KIO_4 were constructed (Figure 6.2) which were used for the estimation and recovery of I^-, IO_3^- and IO_4^- after their separation from mixtures . To determine the lower limit of quantification (LOQ) , different amounts (μg)

of an anion were spotted on TLC plate . chromatographed , area of adsorbent corresponding to the anionic spot was scraped off and then the analyte anions was determined by titrimetry . The lowest amount , below which the chromato-iodometric method is not applicable , is taken as LOQ . The LOQ values for iodide , iodate and periodate are 76.4 , 16.3 and 16.6 μg respectively .

***Recovery from Spiked Water*:** The results presented in Table 6.2 show that the recovery of IO_3^- is always higher than 100% and that of IO_4^- is much lesser than 100 % after 30 min which reduced to zero after 70 min of mixing time of the components . This phenomenon may be explained on the basis of conversion of IO_4^- into IO_3^- . The recovery of I^- was always fairly good . During quantitative determination and recovery of analytes , the relative standard deviation varies from 1.54 to 13.29 pph .

Table 6.1 : hR_F Values of I^- , IO_3^- and IO_4^- with Different Chromatographic Systems .

Anion Studied	Stationary phase	hR_F Values achieved with				
		M_1	M_2	M_3	M_4	M_5
I^-	S_1	65	70	86	90	97
	S_2	56	91	92	92	94
	S_3	95	96	97	97	98
	S_4	90	92	97	98	98
IO_3^-	S_1	0	0	8	24	30
	S_2	0	55	72	85	94
	S_3	89	90	90	92	94
	S_4	0	75	92	90	95
IO_4^-	S_1	0	0	0	0	0
	S_2	0	60	74	85	95
	S_3	*	*	*	*	*
	S_4	0	0	0	0	0

* Badly tailed ($hR_L - hR_T = 100$) spot

Table 6.2: Recovery of Anions from Ternary Mixtures Prepared in DoubleDistilled Water

Anion	Amount Loaded	Recovery after 30 Min of Mixing of Components		Recovery after 70 Min of Mixing of Components	
		Amount Recovered	Relative Recovery	Amount Recovered	Relative Recovery
	(μg)	(μg)	(%)	(μg)	(%)
I^-	152.89	142.35	93.10	131.80	86.21
	305.79	289.39	94.64	273.02	89.28
	458.68	417.44	91.01	407.13	88.76
IO_3^-	32.69	45.01	137.69	55.44	169.60
	65.35	79.22	121.17	89.34	136.65
	98.08	110.79	112.56	116.09	118.38
IO_4^-	33.24	12.95	39.00	ND*	0.00
	66.40	25.59	38.54	ND	0.00
	99.60	38.64	38.81	ND	0.00

* Not detected

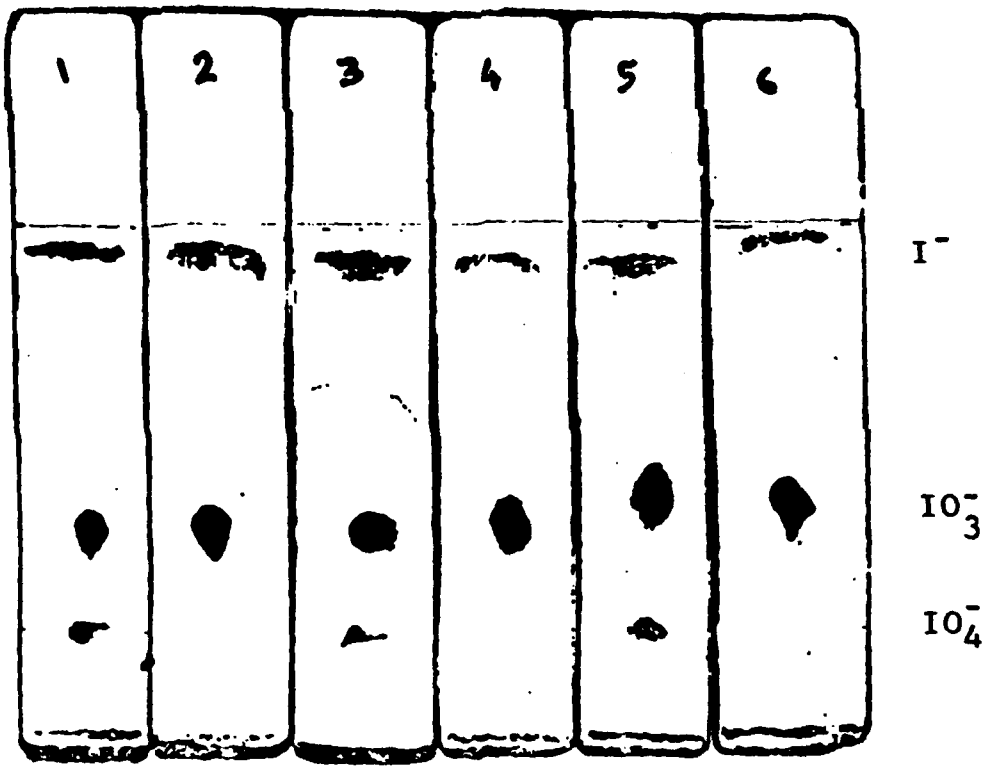


Figure 6.1: TLC Detection of I^-, IO_3^- and IO_4^- under optimized pH and Mixing Time. pH; 4.0 (Plate No. 1,2), 7.0(3,4) and 10.0(5,6); Mixing Time; 30 Min(1), 35 Min(2), 60 Min(3), 65 Min(4), 75 Min(5) and 80 Min(6).

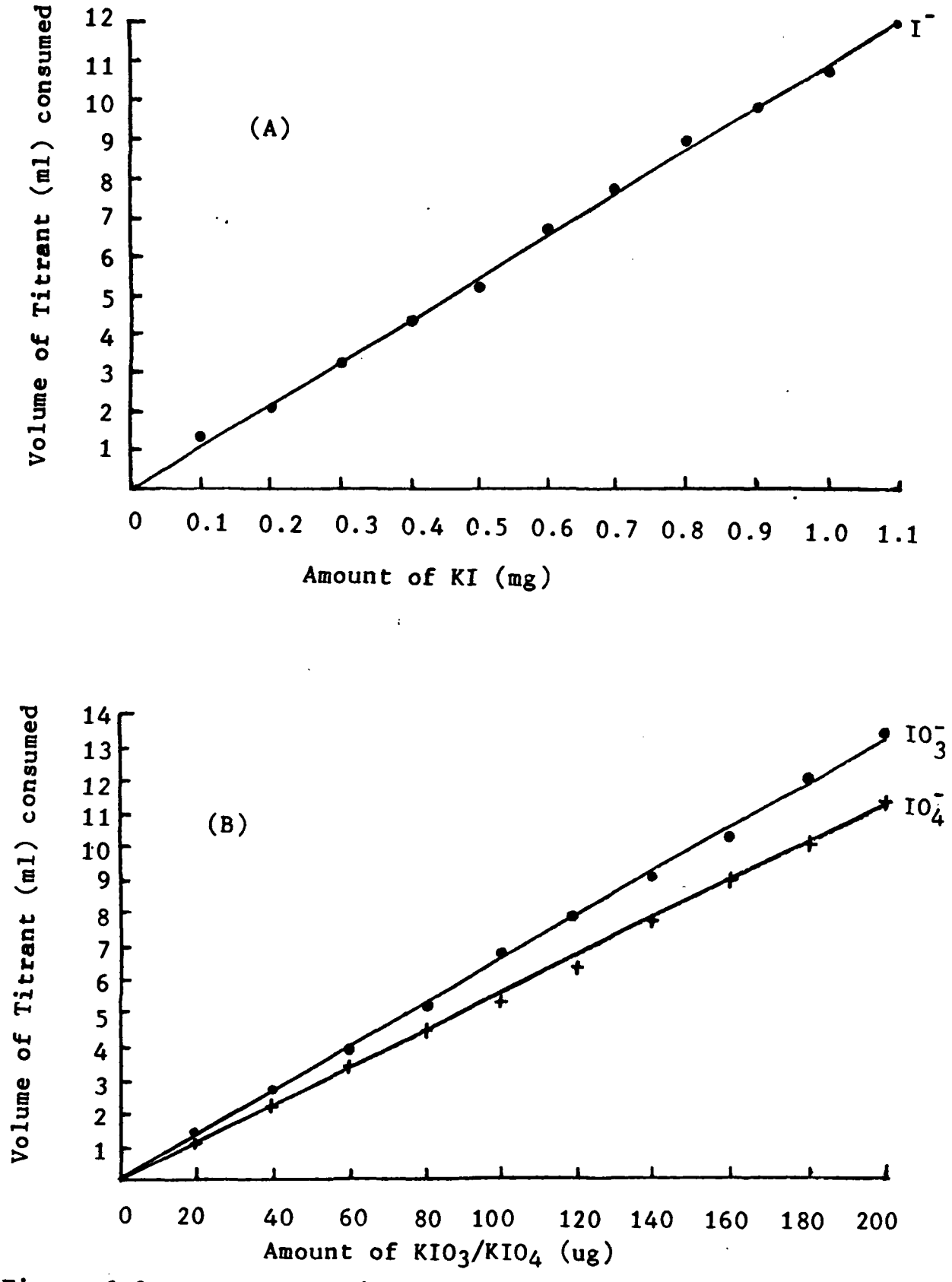


Figure 6.2. Standard Calibration Curves for the TLC-Iodometry of Iodide (A) and its Oxyanions (B).

REFERENCES

1. J.G. Kirchner , in *Thin -Layer Chromatography - Quantitative Environmental and Clinical Applications* , J.C.Touchstone and D.Rogers (Eds.) Wiley - Interscience , New York (1980) 1 .
2. N. Grinberg , *Modern Thin- Layer Chromatography* , Marcel Dekker , New York (1990) 368 .
3. J.Sherma ,*Thin Layer Chromatographic Techniques and Applications*, Marcel Dekker , New York (1994) 146 .
4. A. Mohammad , in *Handbook of Thin -Layer Chromatography* , 2nd edn. ,J. Sherma and B. Fried (Eds.), Marcel Dekker , New York (1996).
5. A. Mohammad and S. Tiwari , *Sep. Sci. Technol.* , 30 (19) (1995) 3577.
6. H. Behrens , *Fresenius J.Anal. Chem.* 236 (1) (1968) 396 .
7. R.Frache , A.Dadone and F.Baffi , *Chromatographia* 7 (1974) 305 .
8. J.Benes ,*Collect. Czech Chem. Commun.* 44 (1979) 1034 .
9. M.H.Hashmi , M.A.Shahid , A.A.Ayaz , F.R.Chughtai , N.Hasan and A.S.Adil , *Anal. Chem.* 38 (11) (1966) 1554 .
10. Y.Maki , *J.Radioanal. Chem.* 27 (1) (1975) 33 .
11. A.Mohammad and S.Tiwari, *Microchem J.* 47 (3) (1993) 379 .
12. J.March , *Advanced Organic Chemistry* , 2nd edn. McGraw-hill , Inc., U.S.A. (1984) 72, 536 .

CHAPTER -VII

COLORIMETRIC DETERMINATION OF SILVER IN ORES WITH PRELIMINARY TLC SEPARATION FROM ASSOCIATED METAL IONS

7.1 INTRODUCTION

In recent years, thin- layer chromatography (TLC) has grown much in status and has enjoyed popularity due to its simplicity , versatility and low cost . TLC with optimization of techniques and layer materials , can be applied for the identification , separation and quantitation of various organic and inorganic species present in pharmaceutical, environmental, geological and biological samples . As a result, it has found interesting applications in the analysis of river , sea and industrial wastewater for heavy metal contents (1-7) ; characterization of hazardous wastes (8) ; determination of heavy metals in water , industrial sewage and aquatic plants (9-11) ; concentration and detection of heavy metal compounds in fresh water (12) ; identification of heavy metals in the human placenta (13) ; separation of heavy metals in tubewell water samples (14) ; recovery of SCN^- in photogenic waste (15) and pesticide residue analysis (16) .

The majority of TLC has been performed on silica gel layers with some use of chemically modified and bonded layers , inorganic ion exchangers , cellulose , silufol , polyamides , chitosan , alumina , cellulose derivatives and mixed sorbent layers .In general, mixtures of organic solvents containing some aqueous acids , bases or a buffer have been found most suitable for the separation of ionic species .

As evident from the literature survey of last twenty- two years (17-19) on TLC of inorganics , few workers have used mixed layers . The binary layers that have been used include silica gel-microcrystalline cellulose (MCC) - NH_4NO_3 , silica gel G- MCC , modified silica gel H- MCC and silica gel-ion exchange gels or Cr (IV) antimonate for cations ; Silica gel -alumina or antimonic acid and kieselguhr - cellulose for anions .

There appears to be no report of using alumina- cellulose mixed layers (plain and impregnated) for the separation of metal ions .

It is heartening that out of more than two hundred references cited on TLC of inorganics and organometallics in a chapter of the Handbook of Thin-Layer Chromatography, only one report refers to the use of alumina mixed with cellulose as layer materials in the analysis of anions (18) .

In continuation of our work on the TLC of inoganics ions on mixed layers (15, 20 -22) , this paper reports a simple and reliable method for the identification , separation and determination of silver .

7.2 EXPERIMENTAL

***Apparatus*:** TLC applicator, plates and jars used were as presented in 3.2 Spectrophotometer (Elico, India, Model SL 171) was used for quantitative studies.

***Chemicals*:** Silver nitrate, ammonia solution (Qualigens); acetone (CDH, India), ammonia (BDH) were used. All other Chemicals used were of Analytical Reagent (AR) grade.

***Test Solutions*:** 1.0% aqueous solutions of following salts were used as test solutions.

(a) Nitrates of Pb^{2+}, Hg^{2+} , Ag^{+}, Tl^{+}, Cd^{2+}, Fe^{3+} and Bi^{3+}

(b) Chlorides of Ni^{2+}, Co^{2+} and Zn^{2+}

(c) Sulfate of Cu^{2+}

Detection Reagent : 8×10^{-3} % (W/V) Dithizone in acetone was used as spray reagent for the visualization of all metal ions studied.

Mobile Phases : Double distilled water ; aqueous solutions (1.0M) of $NaNO_3$, AcONa, $AcONH_4$; and 0.01 - 1.0 M aqueous ammonia were used as eluants.

Stationary Phases : Following plain and mixed layer materials were used as stationary phases.

(a) Alumina G (S_1)

(b) Mixtures of alumina and microcrystalline cellulose in 4:1 (S_2),3:2(S_3) , 1:1 (S_4), 1:2 (S_5) and 1:4 (S_6) ratios .

(c) Microcrystalline cellulose (S_7) .

Procedure :

Preparation of TLC Plates : TLC plates were prepared by adopting the method described in 3.2.

Chromatography : A fixed volume (5 μL) of test solution was spotted on TLC plate at the point of application (3.0 cm above the lower edge), the plate was air dried and then developed with 20 ml of choosen mobile phase in a glass jar. After development of plate by ascending technique upto 10 cm from the point of application, it was air dried and then the analyte substance was visualized as colored spot by spraying the detection reagent on the plate. Each analyte was identified on the basis of its R_F (retardation factor) value.

When two or more metal ions have differential migration (different R_F values) their test solutions were mixed together thoroughly and 10μL, 15μL, and 20μL of binary, ternary or quaternary mixtures was spotted on TLC plate and the chromatography was performed. Separated metal ions on TLC plate were appeared at their original positions. For investigating the effect of inorganic anions (S^{2-},CrO_4^{2-},WO_4^{2-},MoO_4^{2-},I^-, $Fe(CN)_6^{4-}$) on the Ag^+-Cu^{2+} separations , 1% aqueous solution of anion concerned was mixed with binary mixture of $Ag^+$and Cu^{2+} in 1: 2 ratio. In case of complexation, the colored precipitate was dissolved either in ammonia solution or nitric acid.

The clear solution was spotted onto the plate and then chromatographed.

Quantitative studies : Five standard solutions containing concentration of $AgNO_3$ in the range 200 - 1000 ppm were used. 0.1 ml of each solution was spotted onto the TLC plate (stationary phase ; S_4), air dried and then developed with 1.0 M NH_4OH . After air drying the plate, the spot of silver was visualized by spraying the plate with dithizone solution in acetone. The spot was scraped off the plate and then extracted with 8 ml of acetone followed by washing with 2 ml of acetone. The volume of filtrate was made to 10 ml with acetone and the optical density of the filtrate was measured at 480nm (λ max.) to sketch the calibration curve. Each value of optical density taken for the construction of calibration curve was the average of five observations.

Recovery of Silver from Ores : For the synthesis of horn silver(AgCl),Sufficient amount of KCl was added to each aliquot (10 ml) of 0.04, 0.06 or 0.08% $AgNO_3$ solution.To get the complete precipitation of AgCl .The Supernatent was discarded and the white curdy precipitate (Settled down at the bottom of glass tube) was air dried, heated at 50°C for 30 min and then dissolved in 13.36M ammonia (specific gravity, 0.91) . The Solution was made upto 10ml. with 1.0M NH_4OH . 0.1 ml of each solution was spotted onto TLC plate, chromatographed (with S_4 ,1.0M NH_4OH) and quantitatively determined by using the method similar to that for standards.The relative recovery (pph) and the relative standard deviation (pph) were calcuated.

Agentite (Ag_2S) was synthesized by passing H_2S Separately in each solution of 0.04, 0.06 or 0.08% $AgNO_3$. After discarding the supernatent, the resultant black precipitate was dried, digested with concentrated HNO_3 and then diluted upto 10ml with water. Similar to horn silver, three solutions

(Concentration of Ag_2S; 0.04, 0.06 and 0.08%) were prepared, which were chromatographed and colorimetrically determined. Relative recovery and relative standard deviation were calculated for each concentration of sample solution.

7.3 RESULTS AND DISCUSSION :

Qualitative Studies : All experiments were performed at room temperature (20^0c) and carried out atleast in triplicate. The development time for 10 cm ascent was 12-15 min and the results showed good reproducibility. The TLC plates, prepared from mixed adsorbents were found more stable as compared to those coated with alumina. The results of this study are summarized in tables (7.1 - 7.3) and figures (7.1 - 7.3)

Our aim behind this study was to develop a selective TLC - spectrophotometric method for the indentification and quantification of silver ion in the presence of transition metal ions i.e. Fe^{3+} ,Cu^{2+} ,Ni^{2+} ,Co^{2+}, Zn^{2+} , Cd^{2+} , Hg^{2+} , Pb^{2+}, Tl^{+} or Bi^{3+}. The literature survey of twenty- two years (17-19, 23-25) on TLC of inorganic substances showed that Ag^{+} is remained at the point of application in most of the cases and its separation from Fe^{3+} ,Cu^{2+} , Hg^{2+} , Pb^{2+}, Tl^{+} and Bi^{3+} is generally difficult. There are a few chromatographic systems (26 - 30) which allow the maximum migration of Ag^{+}. The Chromatographic system developed by us is a novel system as it allows the rapid and reproducible separation of Ag^{+} from all metal ions studied. Thus, Ag^{+} can be removed from any matrix containing heavy metals e.g. Silver alloyes and geological samples. The selected mobile and stationary phases are simple and inexpensive.

Alumina G (S_1) and microcrystalline cellulose (S_7) were used as stationary phases in conjunction with 1.0M aqueous solutions of $NaNO_3$,

AcONa, $AcONH_4$ or NH_4OH as mobile phase to study the retention behaviour of eleven metal ions. It was observed that metal ions migrate up and showed maximum R_F values on S_7 layer when developed with $NaNO_3$, AcONa or $AcONH_4$. Bi^{3+} is the exception which is strongly retained by stationary phase. The different R_F values of metal ions on $S_7$with 1.0M NH_4OH providing higher separation possibilities. As regards to S_1, all metal ions remain at the point of application except Tl^+ ($hR_F = 20$) when developed with $NaNO_3$ and AcONa. However, Ag^+ showed higher mobility (hR_F=75) on this stationary phase when developed with 1.0M NH_4OH whereas other metal ions remained near the point of application.

In search of better adsorbent, S_1 and S_7 were mixed together in various ratios to get a set of mixed beds (Table 7.1) .Of these stationary phases (S_2 - S_6) ,Cu^{2+} ,Ni^{2+} and Ag^+ give tailed spots on $S_5$and S_6 and no sufficient difference in R_F values of metal ions was achieved on S_2 and S_3. Thus S_2, S_3 ,S_5 and S_6 can not be used for a selective and reproducible separation of metal ions . Out of these six mixed adsorbents, S_4 was found to be the best in terms of detection clarity and selective separation possibilities of metal ions. To find out the optimum concentration of NH_4OH as mobile phase, aqueous solutions containing 0.01 - 1.0 M ammonia were tested. 1.0M NH_4OH was found the most suitable mobile phase for providing differential migration of metal ions (Figure 7.1) . By using S_4 (Stationary Phase) alongwith 1.0M NH_4OH (mobile phase), we achieved experimentally several binary, ternary and quaternary separations (Table 7.2). Effect of acid - base equilibrium (AcOH - NH_4OH) on the mobility of Ag^+ was examined. A peculier behaviour of Ag^+ migration was observed (Figure 7.2). With M_1 and M_2 (AcOH > 50 %) Ag^+ gives a single spot with hR_F value

arround 45 but at M_3 (NH_4OH- AcOH ,1:1) two spots of silver were appeared instead of single spot due to the existence of Ag^+ (hR_F = 45) and diammine argentate ion (hR_F = 70). Double spots formation was also observed with 1.0MAcONH_4 With mobile phases having more then 50%. NH_4OH(M_4 and M_5), a single spot (hR_F = 85) is appeared. It may be attributed to the existence of $Ag(NH_3)_2^+$ species. It appears that below 50% concentration of NH_4OH , silver migrates as Ag^+ ; at 50%, it migrates both as Ag^+ and Ag $(NH_3)_2^+$ species and above 50% the migrating species is the Ag $(NH_3)_2^+$. This observation is important as it highlights the applicability of TLC in studies of chemical speciation .

Quantitative Studies :

Silver (upto 100μg) was spectrophotometically determined using dithizone as chromogenic reagent. Optical densities were measured at 480 nm and plotted against μg amounts of $AgNO_3$to sketch the standard calibration curve (Figure 7.3). This curve was used to study the recovery of Ag (I) from its ores (horn silver and argentite). The results are presented in Table 7.3 . It was observed that the % recovery is below 100 from horn silver (AgCl) while above 100 from argentite (Ag_2S). The later is due to the presence of sulfide ion, as this ion produces colour with dithizone. During entire study. The relative standard deviation remained below 9.09 pph.

Table 7.1: h R_F Values of Metal Ions on Plain and Mixed Sorbent Layers Using 1.0M NH_4OH as Eluant.

Metal Ion	hR_F Values on S_1	S_2	S_3	S_4	S_5	S_6	S_7
Ag^+	75	78	82	90	92	92	95
Tl^+	7	12	15	17	30	45	32
Hg^{2+}	37	35	44	60	72	90	BT
Pb^{2+}	0	0	0	0	0	0	0
Cu^{2+}	12	15	17	20T	BT	BT	BT
Ni^{2+}	10	12	27	45	50	50T	80T
Co^{2+}	0	0	5	10	10	20	22
Zn^{2+}	2	5	10	17	17	36	40
Cd^{2+}	8	13	25	27	43	70	BT
Fe^{3+}	0	0	0	0	0	0	0
Bi^{3+}	0	0	0	0	0	0	0

T; Tailed spot (hR_L - hR_T >30)

BT; Badly Tailed spot (hR_L - hR_T >50)

Table 7.2 : Experimentally Achieved separations of Metal Ions on S_4 Using 1.0M NH_4OH as Eluant. Development Time = 15 min

Type of Mixture	hR_L - hR_T of Separated Ions
Binary	Ag^+ (97 - 70) - Fe^{3+}, Co^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}(34 - 0)
	Ni^{2+}(50 - 20),Cd^{2+}(45 - 15) or Hg^2(62-35)
	Tl^+(24 - 8) - Pb^{2+}, Bi^{3+} (0 - 0) or Hg^{2+} (63 - 40)
	Hg^{2+} (76-54) - Pb^{2+} (0 - 0)
	Ni^{2+} (52 - 24) - Co^{2+} (0 - 0)
Ternary	Ag^+ (95 - 75) - Hg^{2+}(63 - 35) - Pb^{2+} (0 - 0)
	Ag^+(90 - 65) - Ni^{2+} (48 - 25) - Co^{2+} (0 - 0)
	Hg^{2+} (73 - 45) - Tl^+ (25 - 12) - Pb^{2+} (0 - 0)
	Hg^{2+} (72 - 53) - Tl^+ (27 - 12) - Bi^{3+} (0 - 0)
Quaternary	Ag^+ (95-85) - Hg^{2+} (76 - 50) - Tl^+ (27-12) - Pb^{2+} (0 - 0)

Table 7.3: Recovery of Ag (I) from its Ores.

Ore	Amount Loaded (μ g)	Amount Recovered (μ g)	Relative Recovery (%)	Relative S.D* (%)
Horn Silver	25.40	22.28	87.72	9.09
	38.10	35.10	92.12	5.38
	50.80	46.75	92.02	4.83
Argentite	25.40	32.28	127.09	6.58
	38.10	44.18	115.96	3.83
	50.80	52.12	102.60	5.52

*S.D. = Standard Deviation

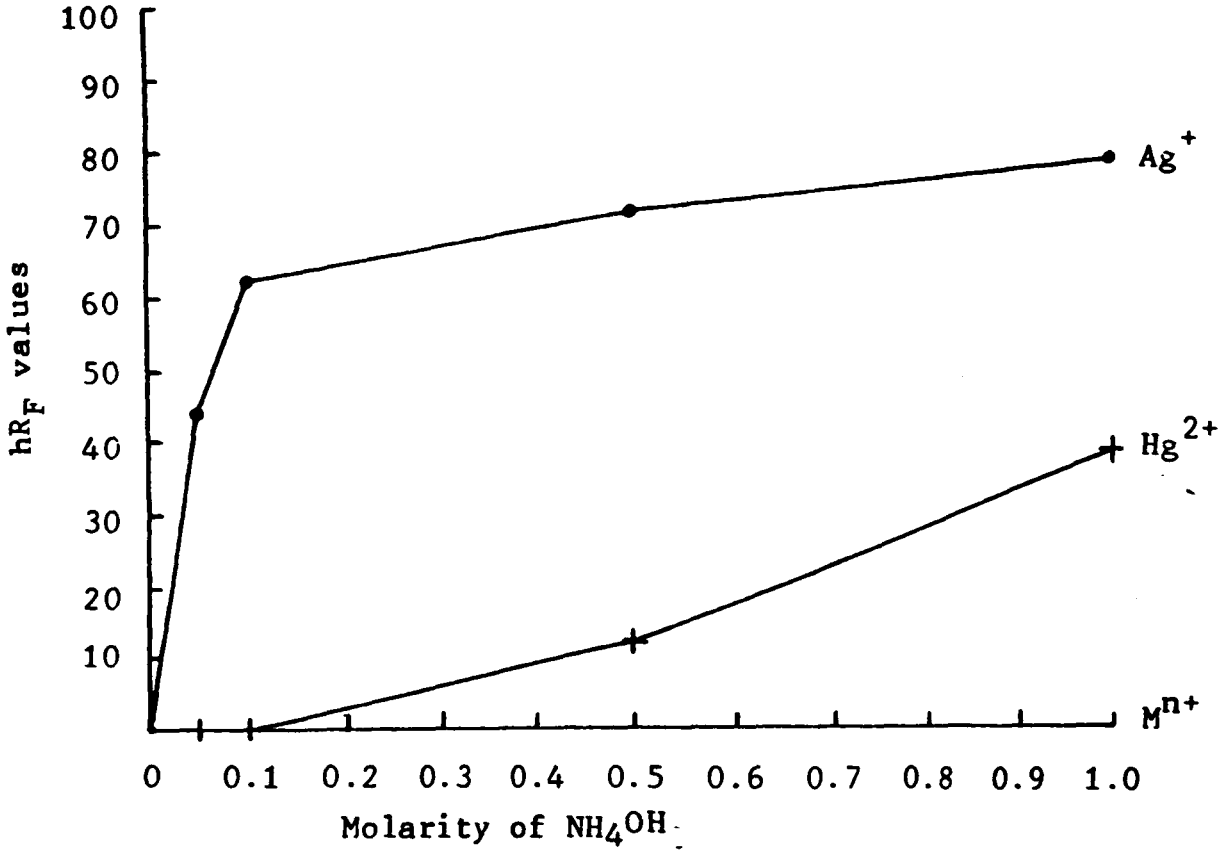


Figure 7.1: Mobility of metal ions on alumina G, under the influence of NH_4OH concentration of mobile phase. All other metal ions remain at the point of application.

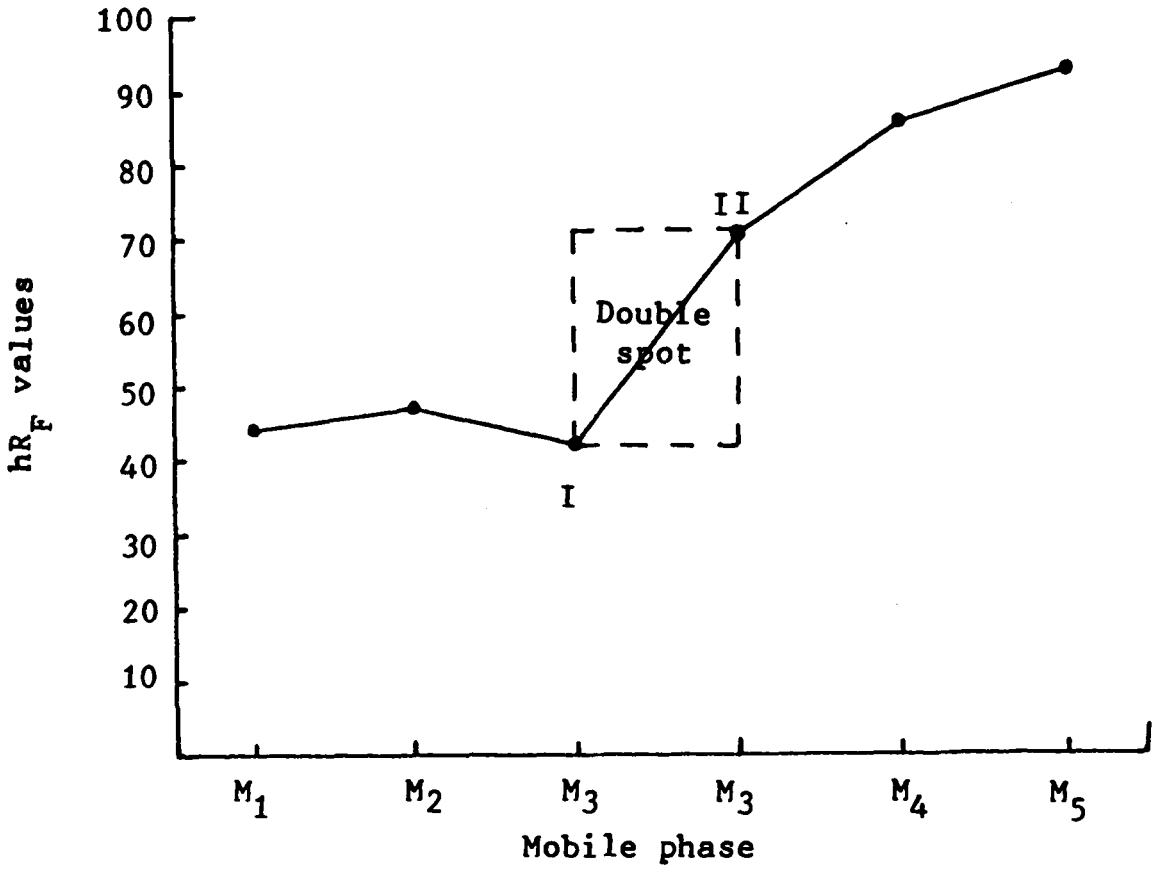


Figure 7.2: Formation of double spot of Ag^+. M_1-M_5 are mixed mobile phases containing AcOH and HH_4OH in 10:0, 7:3, 1:1, 3:7 and 0:10 respectively.

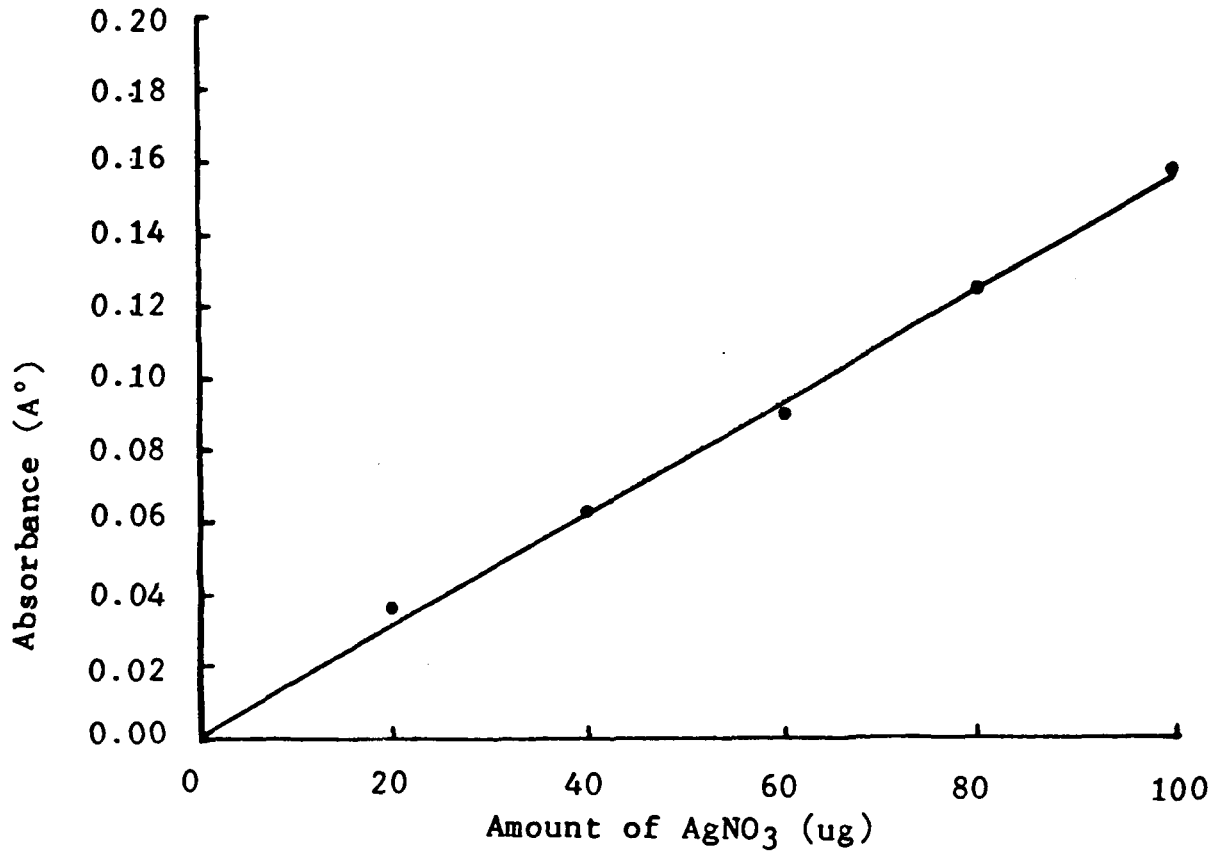


Figure 7.3: Standard calibration curve for the colorimetry of Ag(I).

1. M.P. Volynets, L. P. Kitaeva and A.P. Timerbaev, *Zh. Anal. Khim*, 41 (1986) 1989.

2. G.V. Myasoedova, M.P. Volynets, T.G. Akimova, L.I. Bolshakoua, T.N. Dubrova, S.B. Savvin, A.N. Ermakov, *Zh. Anal. Khim.* 41 (1986) 662.

3. M. Ajmal, A.Mohammad, N.Fatima and A.H.Khan, *Microchem J.* 39 (1989) 361.

4. N.V.N. Kumar and Y.P. Devi, *J. Assoc. Off. Anal. Chem.* 64 (1981) 729.

5. K. Bhagyalakshmi and N.V.N. Kumar, *J.Ind. Pollut. Control* 3 (1987) 57.

6. A. Mohammad and M.A. M. Khan, *J.Chromatogr.* 642 (1993) 455.

7. A. Mohammad, *J.Planar Chromatogr.* 8 (1995) 463.

8. R.D. Stephens and J.J. Chan , in *Thin-Layer Chromatography: Quantitative Environmental and Clinical Applications,*J. C. Touchstone and D.Rogers (Eds.), Wiley, New York (1980) 363.

9. V.N. Kavetskii, A.I. Karnaukhov, and I .M. Pallinko, *Gidrobiol. Zh.* 20 (1984) 65.

10. H. Thielemann, *Acta Hydro chem. Hydrobiol.* 5 (1977) 191.

11. L.Deshmukh and R.B. Kharat , *J.Chromatogr. Sci.* 28 (1990) 400.

12. Y. P. Devi and N.V.N. Kumar, *J. Assoc. Off. Anal. Chem.* 64 (1981) 1301.

13. I. Baranowska, R. Poreba, J.Baranowski and R. Aleksandrowic, *J. Planar Chromatogr.* 5(1992) 469.

14. L.Deshmukh and R.B. Kharat, *Int. J. Environ.Anal. Chem.* 16 (1989) 1.

15. A. Mohammad and J.P.S. Chahar, *J.Chromatogr. A* 774(1997) 373.

16. H. S. Rathore and T.Begum, *J.Chromatogr.* 643(1993) 271.

17. A. Mohammad and K.G. Varshney, in *Handbook of Thin-Layer Chromatography,* 2nd edn., J. Sherma and B. Fried (Eds.), Marcel Dekker, New York (1991) 463.

18. A.Mohammad, in *Handbook of Thin-Layer Chromatography,* J.Sherma and B. Fried (Eds.), Marcel Dekker, New York (1996) 507.

19. A.Mohammad, M.Ajmal, S.Anwar and E. Iraqi, *J. Planar Chromatogr.* 9 (1996) 318.

20. A.Mohammad and S. Tiwari, *Microchem. J.* 47 (1993) 379.

21. A. Mohammad, M. Ajmal, N. Fatima and M. A. M. Khan, *J. Planar Chromatogr.* 5 (1992) 368.

22. A. Mohammad , S.Tiwari and J.P.S. Chahar, *J. Chromatogr. Sci.* 33 (1995) 143.

23. T. Shimizu, K. Hashimoto and K. Tsunoda, *Chromatrographia* 31(1991) 60.

24. J.K. Rozylo, D. Gwis-chomicz and I. Malinowska, *Environ. Protect. Engg.* 16 (1990) 121.

25. A.Mohammad and M. A. M. Khan, *J.Planar Chromatogr.* 8 (1995)134.

26. L.Lepri P.G.Desideri and G. Tanturli, *J.Chromatogr.* 147 (1978) 375.

27. T. Shimizu, H.Kashima and K. Watanabe, *Chromatographia* 11 (1978) 517.

28. T. Shimizu, E. Uehara and M. Ohtani, *Chromatographia* 21(1986) 175.

29. T.Shimizu, F. Nishimura, T.Imai and S. Jindo, *Chem. Environ. Res.* 1 (1992) 73.

30. S. D. Sharma, S. Misra and R. Gupta, *J. Liq. Chromatogr.* 16 (1993) 1833.

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Thin-Layer Chromatographic Separation, Identification, and Determination of Certain Anions

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Abstract

The retention behavior of 19 anions on plain, impregnated, and mixed adsorbent layers of silica gel G, alumina, cellulose microcrystalline, kaolin, and kieselguhr G are examined in aqueous solvent systems. The analytical potential of water, a nontoxic eluant, is explored for its applicability as a simple, inexpensive, and easily available developer in liquid layer chromatography. Better separations of anions are achieved on cellulose with HCl–acetone (1:9) as the eluant and on a cellulose and alumina (1:2) mixed bed with water. The semiquantitative determination of I^-, Br^-, and NO_2^- are attempted. The limits of identification for I^-, Br^-, NO_2^-, SCN^-, BrO_3^-, IO_4^-, IO_3^-, CrO_4^{2-}, and PO_4^{3-} are also determined.

Introduction

Of the various chromatographic techniques, thin layer chromatography (TLC) has recently gained popularity not only because of advancements in technique and instrumentation and improvements in efficiency but also because of its relatively low cost and speed of analysis compared with other highly selective and efficient chromatographic techniques such as high-performance liquid chromatography and gas chromatography. The differential migration of species in TLC is due to varying degrees of affinity of the components in the stationary and mobile phase mixtures. The exact separation mechanisms involved depend on the nature of the two phases and the solutes. Chromatographic retention and selectivity depends upon interactions such as hydrogen bonding and electron pair donor and acceptor, ion–ion, ion–dipole, and van der Waals interactions.

An extensive literature survey (1–10) on TLC was undertaken; it is surprising to note that very little work has been reported on the analysis of anionic mixtures as compared with that of cationic mixtures, as shown in Figure 1. As a continuation of our previous work on the TLC of anions (11–13), this paper describes a simple method for identification, separation, and determination of I^-, SCN^-, NO_2^-, Br^-, BrO_3^-, IO_4^-, IO_3^-, CrO_4^{2-}, and PO_4^{3-} under various experimental conditions.

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Experimental

Reagents

Silica gel G (particle size between 10 and 40 µm; Catalog No. 27335) and methanol were obtained from Glaxo Laboratories (India). Kieselguhr (particle size between 10 and 40 µm; Catalog No. 015036), kaolin (Catalog No. 033059), and cellulose microcrystalline (particle size less than 30 µm; Catalog No. 027984) were obtained from CDH Laboratories (India). All other reagents were of analytical reagent grade.

Test solutions

The test solutions (1% w/v) were sodium salts of nitrate, nitrite, and fluoride and potassium salts of iodide, iodate, periodate, bromide, bromate, permanganate, chromate, dichromate, ferricyanide, ferrocyanide, chloride, phosphate, and sulfite, except thiocyanate and molybdate, which were taken as their ammonium salts. Double distilled water with a specific conductivity ($K = 2 \times 10^{-6}$ ohm^{-1} cm^{-1} at 25°C) was used for the preparation of salt solutions.

Preparation of thin layer plates

Silica gel, alumina, and kieselguhr TLC plates were prepared by mixing the adsorbent with double distilled water in a 1:3 ratio by weight. The resultant slurry was mechanically shaken for 10 min, after which it was applied to well-cleaned 20- × 3.5-cm^2 glass plates with the help of a TLC apparatus (Toshniwal, India) to give a layer of approximately 0.25-mm thickness. The plates were air dried at room temperature and then heated at 100 ± 5°C for 1 h to activate them. After activation, the plates were stored in a desiccator. Cellulose or kaolin coated plates were prepared in a similar fashion by using a slurry made by mixing cellulose or kaolin with double distilled water in a 1:4 ratio by weight. No additional binder was added to the slurry. For impregnated silica gel layers, a slurry

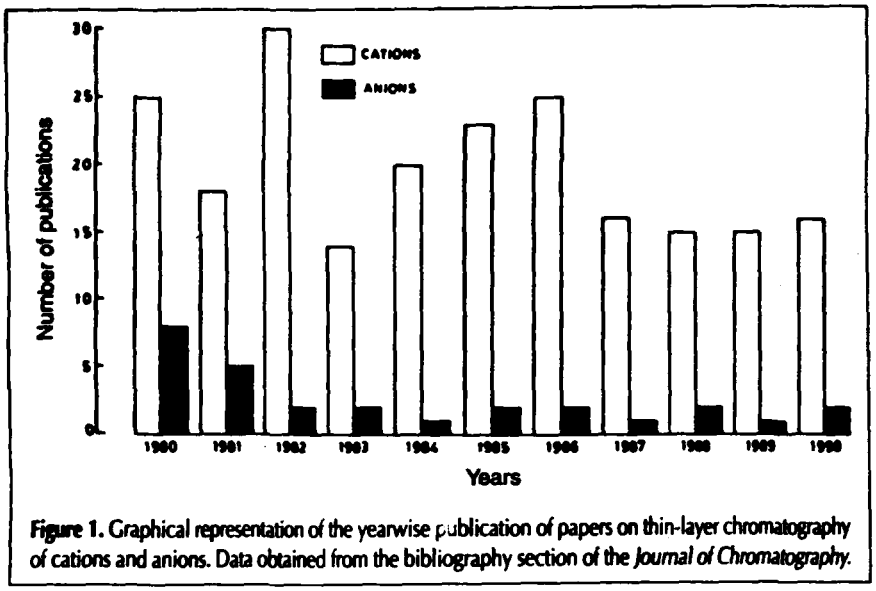

Figure 1. Graphical representation of the yearwise publication of papers on thin-layer chromatography of cations and anions. Data obtained from the bibliography section of the *Journal of Chromatography*.

was made by mixing silica gel with an aqueous solution of 1% $CuSO_4$ in a 1:3 ratio. Thin layers were then prepared as previously described.

Detection reagents

For the detection of various anions, the following reagents were used:

- Saturated $AgNO_3$ solution in methanol for I^-, Br^-, Cl^-, F^-, SO_3^{2-}, CrO_4^{2-}, $Cr_2O_7^{2-}$, and PO_4^{3-}
- Diphenylamine (0.2–0.5%) in 4M H_2SO_4 for NO_2^-, NO_3^-, IO_4^-, IO_3^-, BrO_3^-, MnO_4^-, and WO_4^{2-}
- Ferric chloride (10%) in 2.0M HCl for SCN^-, $Fe(CN)_6^{3-}$, and $Fe(CN)_6^{4-}$
- Alcoholic pyrogallol (0.5%) for MoO_4^{2-} and $Mo_7O_{24}^{6-}$

Mobile phases

The following solvents were investigated: S_1, 0.1M HCl–acetone (1:9); S_2, 1.0M formic acid; S_3, 1.0M sodium formate; S_4, double distilled water.

Stationary phases

The following sorbent layers were used: A_1, silica gel impregnated with aqueous 1% $CuSO_4$ solution; A_2, silica gel G; A_3, alumina; A_4, cellulose microcrystalline; A_5, kaolin; A_6, kieselguhr G; A_7, alumina + cellulose (2:1); A_8, kieselguhr + cellulose (1:2, 2:1).

Procedure

A sample volume (1.0–10 µL) containing a sufficient amount of analyte (0.1–10 µg) was applied using a micropipette about 2.0 cm above the lower edge of the chromatoplates. The spots were dried, and the plates were developed in glass jars (24 × 6 cm^2) containing the mobile phase using a one-dimensional ascending technique. Before developing the plates, the glass jars that contained the mobile phase were covered with a lid for about 20 min so that the glass jars would get presaturated with the mobile phase vapors. The mobile phase (solvent) was allowed to migrate up to 10 cm from the starting line in all cases. After development, the plates were dried at room temperature, and the anion spots were visualized using the appropriate spray reagent (Figure 2).

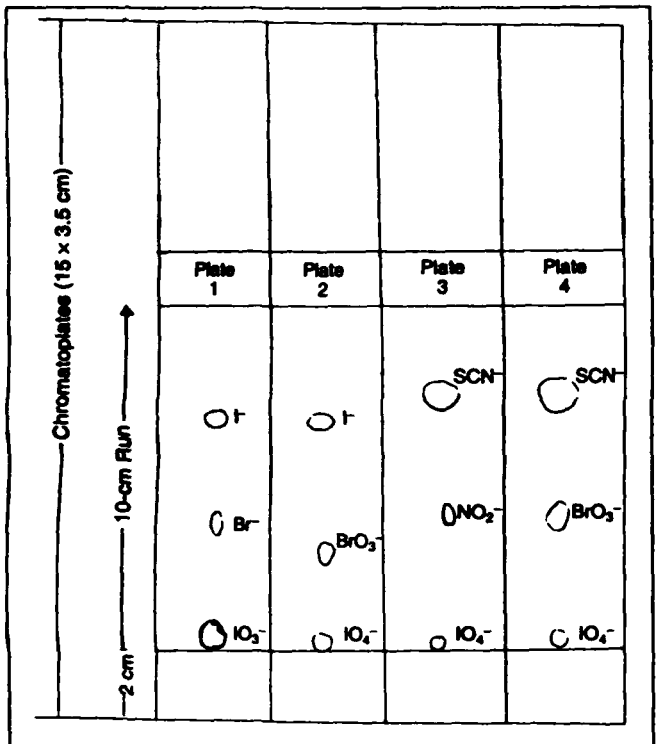

Figure 2. Diagram of some ternary separations: 1, IO_3^- –BR^-–I^-; 2, IO_4^- –BRO_3^- –I^-; 3, IO_4^- –NO_2^- –SCN^-; 4, IO_4^- –BrO_3^- –SCN^-. Conditions: stationary phase, cellulose; mobile phase, HCl–acetone (1:9).

Table I. R_f Ranges (sample size, n = 3) Involved in Ternary Separations on Cellulose Microcrystalline Thin Layers Using 0.1M HCl–Acetone (1:9) as Eluant[a]

Anions separated	Lower detection limit (µg)	R_f	Accompanying anions
I^-	7.64	0.66 – 0.75	IO_4^-,IO_3^-,NO_2^-,Br^-,BrO_3^-, PO_4^{3-},CrO_4^{2-}
SCN^-	3.81	0.73 – 0.78	IO_4^-,IO_3^-,NO_2^-,BrO_3^-, PO_4^{3-},CrO_4^{2-}
NO_2^-	3.33	0.35 – 0.41	IO_4^-,IO_3^-,SCN^-,Br^-,I^-
Br^-	6.71	0.37 – 0.38	IO_4^-,IO_3^-,I^-
BrO_3^-	3.83	0.27 – 0.32	IO_4^-,IO_3^-,SCN^-,I^-
PO_4^{3-}	11.58	0.32 – 0.38	IO_4^-,IO_3^-,SCN^-,I^-
CrO_4^{2-}	4.06	0.35 – 0.42	IO_4^-,SCN^-,Br^-,I^-
IO_4^-	4.15	0.04 – 0.06	I^-,SCN^-,NO_2^-,Br^-,BrO_3^-, PO_4^{3-},CrO_4^{2-}
IO_3^-	4.08	0.04 – 0.07	I^-,SCN^-,NO_2^-,Br^-,BrO_3^-,PO_4^{3-}

[a] Standard R_f values of individual ions: I^-, 0.73; SCN^-, 0.83; NO_2^-, 0.41; Br^-, 0.45; BrO_3^-, 0.40; IO_4^-, 0.07; IO_3^-, 0.08; CrO_4^{2-}, 0.42; PO_4^{3-}, 0.43.

For semiquantitative determination of I^-, Br^-, and NO_2^-, 0.01 mL of various standard solutions of KI, KBr, and $NaNO_2$ (2.5–40%) were spotted on silica gel impregnated with a 1% $CuSO_4$ solution. The chromatoplates were developed with S_1. After detection, the spot was copied onto tracing paper from the chromatoplates, and then the spot area was calculated graphically. Spot areas reported in this paper represent the averages of triplicate tests.

The limit of detection of various anions was determined by spotting different amounts of anionic solutions on the chromatoplates. The plates were developed, and the spots were detected as described above. The method was repeated with successive lowering of the amount of anion until spots could no longer be detected. The minimum amount of anion that was able to be detected was taken as the limit of detection.

A volumetric procedure was applied for the quantitative determination of iodate after its TLC separation from periodate. A standard volumetric method (14) was set up using 0.01M sodium thiosulfate solution as an intermediate solution. For the determination of IO_3^- in the presence of IO_4^-, various samples containing a mixture of KIO_3 and KIO_4 in variable amounts were prepared. Using a lambda pipette, 0.01 mL of the resultant mixture was loaded on the chromatoplates. The plates were developed with double distilled water (15). A pilot plate was employed simultaneously in order to locate the exact position of the spot on the working plate. The area occupied by IO_3^- was scraped, and IO_3^- was extracted with 1.0M HCl. The adsorbent was separated from the solution by filtration and washed with 1.0M HCl to ensure complete extraction of IO_3^-. The filtrate was added to a blank that was prepared by mixing 2 mL KI (1%), 2 mL concentrated HCl, and 0.2 mL KIO_3 (1%). The contents were titrated with a 0.01M $Na_2S_2O_3$ solution, the blank was also simultaneously titrated with 0.01M sodium thiosulfate, and the difference between the volume of the $Na_2S_2O_3$ solution consumed in both cases was taken for the determination of IO_3^- in the sample.

Table II. Separations Achieved Experimentally on Different Sorbent Layers Using Distilled Water as Eluant

Sorbent system	Separations (R_L–R_T)*
Silica gel 'G'	IO_4^-(0.00) – IO_3^-, BrO_3^-, NO_2^-, I^-, WO_4^{2-}, $Fe(CN)_6^{3-}$, or $Fe(CN)_6^{4-}$ (1.0 – 0.85)
Alumina	NO_3^- (1.0 –0.82) – WO_4^{2-}, MnO_4^-, PO_4^{3-} (0.00) NO_3^- (1.0 –0.82) – CrO_4^{2-} or $Cr_2O_7^{2-}$ (0.18–0.00) NO_3^- (1.0 –0.86) – $Fe(CN)_6^{3-}$ (0.1–0.00) SCN^- (1.0 –0.86) – CrO_4^{2-} or $Cr_2O_7^{2-}$ (0.19–0.00)
Cellulose	MnO_4^- (0.00) – IO_4^-, IO_3^-, CrO_4^{2-} or $Cr_2O_7^{2}$ (1.0-0.90)
Alumina + Cellulose (2:1)	MnO_4^- (0.00 –0.00) – IO_3^- (0.45–0.30) – NO_2^- (1.0–0.89) MnO_4^- (0.00 –0.00) – IO_3^- (0.41–0.24) – BrO_3^- (1.0–0.86)

* R_L is the R_f of the leading front and R_T is the R_f of the trailing front of the spot.

Table III. Determination of Iodate with Preliminary TLC Separation from Periodate on Silica Gel G Layers with Distilled Water as the Eluant

Amount of IO_4^- taken (mg)	Amount of IO_3^- loaded (mg)	Amount of IO_3^- recovered* (mg)	Error (%)	Relative standard deviation (ppt)
0.415	0.405	0.412	–1.7	18.1634
0.365	0.450	0.441	+2.0	15.3794
0.307	0.500	0.489	+2.2	13.0410
0.232	0.590	0.601	–1.9	12.6718

* Each value is the average of five determinations (n = 5).

Results and Discussion

Tables I and II show that the chromatographic systems can be successfully applied for the separation of several anions from their binary and ternary mixtures. The results in Table I indicate that well-resolved ternary separations of IO_4^- and IO_3^- can be realized from a synthetic mixture containing I^- or SCN^- and NO_2^-, Br^-, BrO_3^-, CrO_4^{2-}, or PO_4^{3-}. The proposed method is well-suited for microgram detection of anions on cellulose microcrystalline layers. It is clear from Table II that distilled water can also be used as a nontoxic eluant for some binary separations of anions. In addition to qualitative separations, quantitative and semiquantitative determination of certain anions with preliminary separation on thin layers can also be made. Table III presents the results of quantitative determination of iodate in the presence of periodate. It is evident from Table III that the proposed method is accurate (percent error, ± 2.2) and reproducible (relative standard deviation, 18 ppt).

We attempted to semiquantitatively determine IO_4^-, IO_3^-, NO_2^-, Br^-, and I^- by measuring the size of the spots. We outlined the spots on a piece of paper and determined the weight of the paper strip covering the spot area. A linear relationship was obtained when the weight of the paper covering the spot area was plotted against the area of the spot (Figure 3). The linearity was maintained up to 160 mg of NO_2^-, 80 mg of I^-, and 100 mg of Br^-. The deviation from linearity started if the loading amount exceeded the upper limit. The reproducibility of data plotted in Figure 3 was checked by two independent analysts. The values obtained by both the analysts differ by ±15% from the average value as plotted in Figure 3. Thus, the method can safely be applied for semiquantitative determination of Br^-, I^-, and NO_2^-. However, the semiquantitative method was unsuitable for IO_3^- and IO_4^- because of the lower solubility of KIO_3 or KIO_4 in water.

A plot of loading amount versus spot area for NO_2^-, Br^-, and I^- follows the equation $y = mx + c$, where c has positive values. However, a plot of c/m values versus atomic weight/molecular weight of anion/anionic salt shows a linear relationship (Figure 4). Thus c/m is the molecular weight of the anion salt or the molecular weight of the anion salt times a constant.

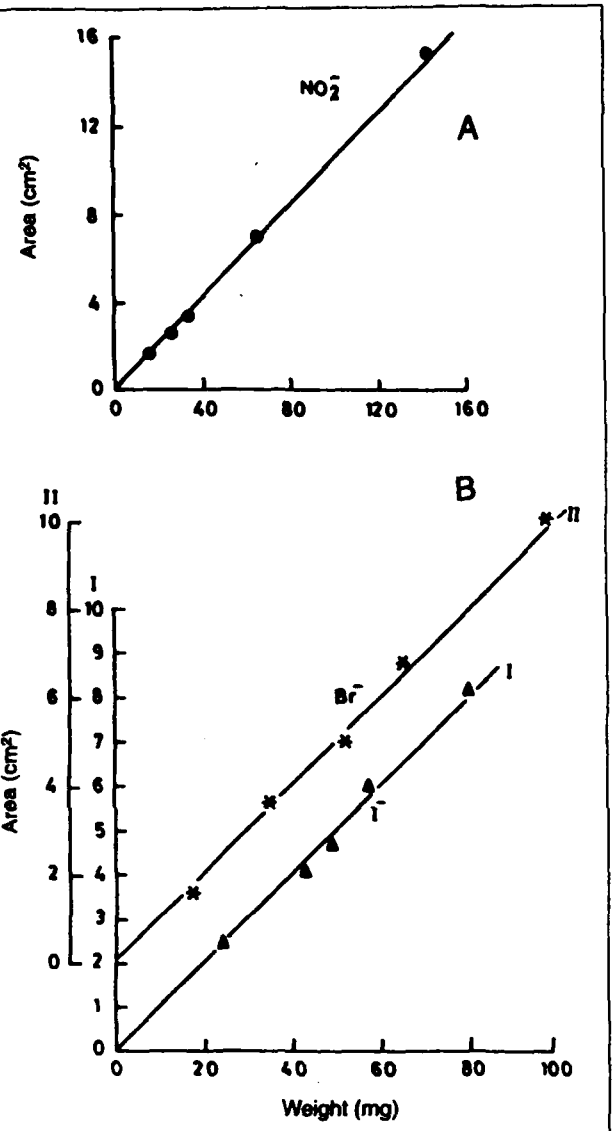

Figure 3. Plot of spot area versus weight of paper covering the spot area. Conditions: stationary phase, silica gel impregnated with 1% $CuSO_4$; eluant, HCl–acetone (1:9).

This relationship may be useful to correlate the spot area with the atomic weight of anions or molecular weight of anionic salts.

Conclusion

The chromatographic system consisting of cellulose thin layers and 0.1M HCl–acetone (1:9) as the eluant is the most suitable system for the identification, separation, and quantitation of several anions. The determination of IO_3^- with preliminary separation from IO_4^- is important, as iodate is reduced to iodide in alkaline medium.

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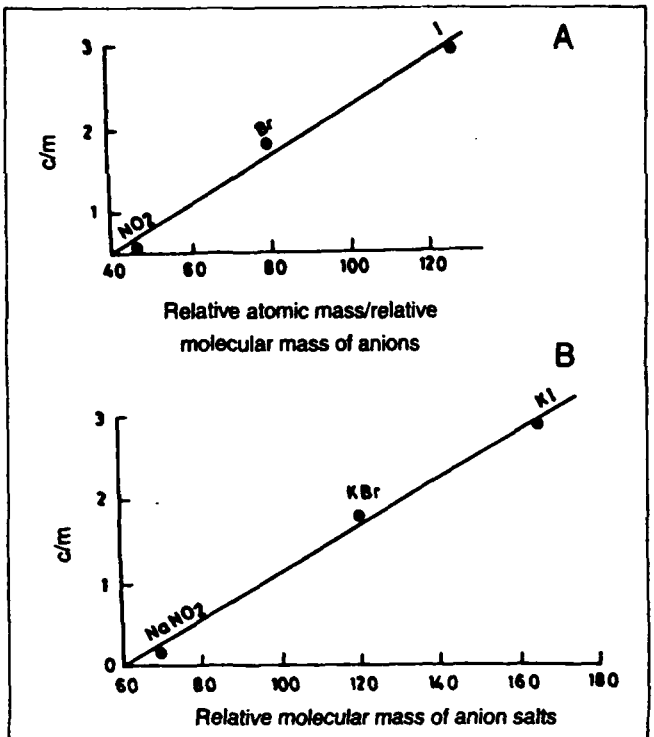

Figure 4. Plot of *c/m* values versus atomic weight/molecular weight of anion/anionic salt. Conditions: stationary phase, silica gel impregnated with 1% $CuSO_4$; eluant, HCl–acetone (1:9).

References

1. J.C. Kirchner. *In Thin-Layer Chromatography: Quantitative, Environmental and Clinical Applications.* J.C. Touchstone and D. Rogers, Eds. Wiley Interscience, New York, NY, 1980.
2. E. Stahl. In *Thin-Layer Chromatography,* 2nd ed. E. Stahl, Ed. Springer Verlag, Berlin, Germany, 1969.
3. *Handbook of Thin-Layer Chromatography.* J. Sherma and B. Fried, Eds. Marcel Dekker, New York, NY, 1990.
4. R. Kuroda and M.P. Volynets. In *CRC Handbook of Chromatography: Inorganics,* Vol I. M. Qureshi, Ed. CRC Press, Boca Raton, FL, 1987.
5. A. Mohammad and M.A. Majid Khan. Novel applications of thin-layer chromatography in inorganic analysis. *J. Chem. Environ. Res.* **1(2):** 3–31 (1992).
6. A. Mohammad and N. Fatima. Cation Thin-layer chromatography in mixed organic solvents containing s-butylamine; Separation of Zn (II) from Cd (II) and Cu (II). *Chromatographia* **23:** 653–56 (1987).
7. U.A.T. Brinkman, G. de Vries, and R. Kuroda. Thin-layer chromatographic data for inorganic substances. *J. Chromatogr.* **85:** 187–326 (1973).
8. J. Sherma. Thin-layer and paper chromatography. *Anal. Chem.* **58:** 69R–81R (1986).
9. J. Sherma. Thin-layer and paper chromatography. *Anal. Chem.* **60:** 74R–86R (1988).
10. J. Sherma. Planar chromatography. *Anal. Chem.* **64:** 134R–47R (1992).
11. A. Mohammad and S. Tiwari. Thin-layer chromatographic separation and identification of some anions on copper sulphate impregnated silica gel layers. *Chromatographia* **30:** 405–409 (1990).

12. A. Mohammad and S. Tiwari. Chromatography of anionic pollutants on silica gel layers: Selective microgram separation of NO_2^- and NO_3^-. *Microchem. J.* **44**: 39–48 (1991).
13. A. Mohammad and S. Tiwari. *J. Planar Chromatogr. Mod. TLC.* **4**: 485–87 (1991).
14. W.W. Scot. Effect of heavy metals on the chromatographic separation of periodate from iodate, bromate, iodide, molybdate and ferrocyanide. In *Standard Methods of Chemical Analysis*, 5th ed. D. Van Nostrand, New York, NY, 1939.
15. A. Mohammad and S. Tiwari. Identification and separation of some anions on plain and mixed adsorbent layers using water as eluant. *Microchem. J.* **47**: 379–85 (1993).

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Water-in-Oil Microemulsion as Mobile Phase in Thin-Layer Chromatographic Retention Studies of Anions

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ABSTRACT: A water-in-oil (W/O) microemulsion, consisting of sodium dodecyl sulphate (SDS)/1-pentanol/water/heptane, has been used as mobile phase for the separation of inorganic anions from their binary mixtures, for example, IO_4^--NO_2^-, IO_4^--BrO_3^-, IO_4^--I^-, MnO_4^--BrO_3^-, MnO_4^--NO_2^-, and MnO_4^--Br^-. The weight ratio, SDS/*n*-pentanol was kept constant at 1/2.46 for all compositions. The retention efficiency of anions on layers of silica gel G, alumina, microcrystalline cellulose, kieselguhr G, and mixtures of kieselguhr and cellulose in 4:1 and 3:2 ratios has been examined with the W/O microemulsion system as a mobile phase. Thin layers of kieselguhr were most useful for differential migration of anions. Quantitative separation of IO_4^- from accompanying ions, limits of identification, and dilution of few anions are reported. The effects of amines, phenols, and heavy metals on the separation efficacy of IO_4^- also have been investigated.
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KEY WORDS: Detection, identification, inorganic anions, kieselguhr adsorbent, microemulsion, peak height, periodate determination, SDS, separation, TLC.

Microemulsions are isotropic, clear, or translucent thermodynamically stable dispersions of oil, water, surfactant, and often a small amphiphilic molecule, called a cosurfactant (1). The droplet diameters in microemulsions range from 100 to 1000 Å. The microemulsion systems are usually transparent because of their much smaller droplet size compared to macroemulsions. One can distinguish three different types of microemulsions: Oil microdroplets enclosed in a surfactant–cosurfactant film are dispersed in an aqueous continuous phase (oil-in-water, O/W type); water droplets are dispersed in the oil-continuous phase (water-in-oil, W/O type); and if oil and water droplets overlap in each other, the system is called bicontinuous (2). W/O microemulsions are complex fluids that have wide-ranging applications, for example, as novel lubricants, reaction media for new synthetic chemistry, and mobile phases in separation science (3,4). Since the first report by Armstrong and Henry (5), micellar chromatography has been a subject of interest to many analytical chemists (6–8). It recently has been shown that W/O microemulsions could be used as normal liquid chromatographic mobile phases (9). Interesting selectivities, along with poor efficiencies, were obtained. It must be noted that the W/O microemulsions used did not contain any alcohol cosurfactants. It was reported earlier that the addition of 1-pentanol to the mobile phase greatly improves efficiencies by reducing the adsorbed amount of emulsifier (surfactant). In this work, a W/O microemulsion with 1-pentanol as cosurfactant was used as eluant because use of such phases in thin-layer chromatography (TLC) is an unexplored area of research. In these systems, water microdroplets, enclosed in surfactant/cosurfactant film, are dispersed in an oil-continuous phase (10). One characteristic feature of microemulsions is their wide domain of existence in the pseudoternary phase diagram (9,11).

In continuation of our earlier studies on TLC of anions (12–14), this report describes simple methodology for identification and separation of anions under optimal experimental conditions with a W/O microemulsion as novel eluant. The use of such phases may offer unique solutions for the change in retention data, due to the influence of water on the stationary phase (15) because water is present in the core of W/O microemulsion droplets.

EXPERIMENTAL PROCEDURES

Chemicals. Specially pure sodium dodecyl sulphate (SDS) and *n*-heptane (99%) were obtained from BDH (Poole, United Kingdom), and *n*-pentanol was a Riedel product (99%). Demineralized water, redistilled from alkaline potassium permanganate, was used. Alumina, microcrystalline cellulose, and kieselguhr were obtained from CDH Laboratories (India), while silica gel G was obtained from Glaxo (India). All other chemicals (Analar grade) were used as supplied.

Test solutions. The test solutions (1%) were either Na or K salts of all anions studied, except SCN^-, which was used as ammonium thiocyanate. Solutions of the nitrates of lead, silver, and bismuth, and the chloride of mercury were prepared in demineralized water, which contained small quantities of the corresponding acid to limit the extent of hydrolysis. The

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solutions (1%) of various amines and phenols used were prepared in methanol.

Chromatography. A TLC apparatus (Toshniwal, India) was used for the preparation of 20 × 3.5 cm^2 glass plates. The chromatography was performed in 24 × 6 cm^2 glass jars.

Detectors. The following spray reagents were used for the detection of various anions. (i) Saturated $AgNO_3$ solution in methanol for I^-, Br^-, Cl^-, F^-, CrO_4^{2-}, $Cr_2O_7^{2-}$, SO_3^{2-}, and PO_4^{3-}; (ii) diphenylamine (0.2—0.5%) in 4M H_2SO_4 for NO_2^-, NO_3^-, IO_4^-, BrO_3^-, MnO_4^-, and WO_4^{2-}; (iii) ferric chloride (10%) in 2M HCl for SCN^-, $Fe(CN)_6^{3-}$, and $Fe(CN)_6^{4-}$; (iv) alcoholic pyrogallol (0.5%) solution for MoO_4^{2-} and $Mo_7O_{24}^{6-}$.

Chromatographic systems. The following sorbent layers were used as stationary phases: S_1 = silica gel G; S_2 = alumina; S_3 = microcrystalline cellulose; S_4 = kieselguhr G; S_5 = kieselguhr + cellulose (4:1), (3:2).

Mobile phase. The W/O microemulsion, used as mobile phase, was prepared by titrating a coarse emulsion of *n*-heptane (160 mL), water (8 mL), and SDS (8 g) with *n*-pentanol (24 mL). Heptane was chosen for the oil phase becaue of low toxicity of the odd-carbon number alkanes. The microemulsion was produced at 30°C.

Preparation of TLC plates. The TLC plates were prepared by mixing adsorbents with demineralized water in a 1:3 ratio by weight (1:4 ratio for S_3). The resultant slurry was mechanically shaken for 10 min, after which it was applied on well-cleaned glass plates to give a uniform layer of 0.25 mm thickness. The plates were air dried at room temperature and then heated at 100 ± 5°C for 1 h. After activation, the plates were stored in an air-tight chamber. No binder was added during the preparation of plates.

Procedure. A sample volume (1.0–10 µL) with a sufficient amount of analyte (0.1–10 µg) was applied with the aid of micropipette about 2.0 cm above the lower edge of the TLC plate. The spots were dried, and the plates were developed by the one-dimensional ascending technique, in glass jars (24 × 6 cm^2) that contained the mobile phase. Before the development of plates, the glass jars, containing mobile phase, were covered with a lid for about 20 min, so that the glass jars may get presaturated with the mobile phase vapors. The solvent was allowed to migrate up to 10 cm from the starting line in all experiments. After development, plates were dried at room temperature, and the spots were visualized with the appropriate spraying reagent.

The identification limits of various anions were determined by spotting different amounts of anionic solutions on the chromatoplates. The plates were developed, and the spots were detected as described above. The method was repeated with successive lowering of the amount of anion until spots could no longer be detected. The minimum amount of anion just detectable was taken as the limit of detection.

RESULTS AND DISCUSSION

We have tried identification and separation of anions on various adsorbents (S_1–S_5). Kieselguhr thin layers were highly effective for differential migration of anions. Table 1 summarizes some binary separations of IO_4^- and MnO_4^- from accompanying anions by using the W/O microemulsion as mobile phase and kieselguhr as the stationary phase. Separations achieved are well resolved and compact in this chromatographic system. It is an important aspect of this study because our past experience (14) has shown that kieselguhr, being only slightly active, fails to provide any fruitful data when used as stationary phase for normal TLC of anions with organic or mixed aqueous-organic solvent systems. This indicates that the W/O microemulsion plays a crucial role in enhancing the differential migration of anions on kieselguhr. This is possibly due to the presence of a water core in W/O microemulsion droplets which provides a restricted volume (16) and are responsible for specific selectivities. The hR_F values of separated anions are given in Table 1. The R_F value of each individual anion changes slightly when it is chromatographed in mixture with other anions.

TABLE 1
Binary Separations Achieved Experimentally with Water-in-Oil Microemulsion[a]

Stationary phase	Separations (hR_F)
Kieselguhr G	IO_4^- (5.75)-NO_2^- (89.5)
	IO_4^- (6.75)-BrO_3^- (89.25)
	IO_4^- (7.25)-I^- (88.25)
	IO_4^- (7.5)-Br^- (94.0)
	MnO_4^- (5.0)-BrO_3^- (91.0)
	MnO_4^- (5.0)-NO_2^- (90.0)
	MnO_4^- (2.5)-Br^- (93.25)

[a]Note: $hR_F = R_F \times 100$, $R_F = (R_L + R_T)/2$. R_L = R_F of leading front of spot. R_T = R_F of trailing front of spot.

Table 2 presents the detection and dilution limits of anions. The proposed method is highly sensitive for most ions, except Br^-, I^-, and IO_4^-. The most interesting behavior is exhibited by MnO_4^-, which can be detected easily, even if it is present at 1 µg. Similarly, CrO_4^{2-} and BrO_3^- can be detected easily. This may well be attributed to the fact that, in W/O microemulsion, these solutes are localized in the hydrophilic core, which is responsible for some sort of preconcentration

TABLE 2
Detection and Dilution Limits of Anions as Their Salts on Kieselguhr Layers Developed with Water-in-Oil Microemulsion

Anion	Lower detection limit (µg)	Dilution limit[a]
IO_4^-	20	1:1000
IO_3^-	10	1:2000
BrO_3^-	2	1:10,000
I^-	20	1:1000
Br^-	20	1:1000
MnO_4^-	1	1:20,000
CrO_4^{2-}	2	1:10,000

[a]Dilution limit = 1:(volume of test solution × 10^6)/[limit of detection (µg)].

TABLE 3
Quantitative Separation of IO_4^- (20–100 µg) from Milligram Amounts of NO_2^-, Br^-, and I^- with Chromatographic System as in Table 1

Amount of accompanying anion salt (mg)		Separations (R_L-R_T)
$NaNO_2$	1.25	IO_4^- (0.15–0.00)-NO_2^- (1.0–0.46)
KBr[a]	1.25	IO_4^- (0.11–0.00)-Br^- (1.0–0.76)
	2.5	IO_4^- (0.10–0.00)-Br^- (1.0–0.68)
	5.0	IO_4^- (0.10–0.00)-Br^- (1.0–0.48)
KI	1.0	IO_4^- (ND[b])-I^- (1.0–0.70)
	2.0	IO_4^- (ND-I^- (1.0–0.65)
	4.0	IO_4^- (ND)-I^- (1.0–0.20)

[a]Spots of Br^- appear after 20 min. [b]ND = not detected.

of anions (17). Further, the anionic charge on the microemulsion droplet is responsible for fast transfer of anionic species out of the microemulsion droplet and easily available to interact with the detection reagents.

Table 3 presents some quantitative separations of anions. KBr, up to 5 mg, and $NaNO_2$, up to 1.25 mg, can be easily separated from KIO_4 (100 µg). Separation of KIO_4 from milligram quantities of KI does not take place, KIO_4 remains undetected while KI produces a tailed spot. Thus, increasing the KI amount produces a deleterious effect and causes poor or no detection of KIO_4. This possibly may be due to reduction of KIO_4 to iodine in solutions of moderate acidity. Because of this fact, we carried out the separation of IO_4^- from NO_2^-, Br^-, and I^-.

Finally, Table 4 summarizes the effect of various additives on the separation efficacy of IO_4^- from Br^- and NO_2^-. Amines by and large do not influence the separations, but there are drastic changes in the R_F values of ions, compared with their standard R_F values. The separation mixture precipitates by the addition of amines. In case of β-napthylamine, Br^- remains undetected because the entire spot (R_F range of Br^-) is superimposed by the solvent uptake of β-napthylamine (deep purple color spot). The situation for NO_2^- is similar, but the spot of NO_2^- is clear. Spots of Br^-, when chromatographed with diphenylamine and 2-nitroaniline, appeared as streaks after one-half hour. Phenols neither hamper separations nor produce precipitation. However, addition of pyrogallol produces slight turbidity, and IO_4^- remains undetected when separated from NO_2^- or Br^-. In this case, the R_F range of IO_4^- gets superimposed by pyrogallol. Heavy metals also do not hinder the separations except silver, which causes dense precipitation as a result of which Br^- remains undetected. Addition of lead to the separation mixture of IO_4^- and Br^- leads to poor detection of Br^-. Mercury shows no effect on the separation of IO_4^- from Br^- or NO_2^-.

An attempt has been made for the semiquantitative determination of IO_4^- by employing a peak height measurement procedure. For this purpose, 0.1 mL standard aqueous solu-

TABLE 4
Effect of Additives on Some Binary Separations of IO_4^- from NO_2^- and Br^-[a]

Additives	Separations R_F)
1. Amines	
(a) β-Napthylamine	IO_4^- (0.05)-NO_2^- (0.85)
	IO_4^- (0.06)-Br^- (ND[b])
(b) Diphenylamine	IO_4^- (0.21)-NO_2^- (0.88)
	IO_4^- (0.21)-Br^- (0.74)
(c) 2-Nitroaniline	IO_4^- (0.20)-NO_2^- (0.65)
	IO_4^- (0.17)-Br^- (0.78)
2. Phenols	
(a) Phenol	IO_4^- (0.03)-NO_2^- (0.82)
	IO_4^- (0.10)-Br^- (0.85)
(b) Resorcinol	IO_4^- (0.07)-NO_2^- (0.90)
	IO_4^- (0.07)-Br^- (0.81)
(c) Pyrogallol	IO_4^- (ND)-NO_2^- (0.80)
	IO_4^- (ND)-Br^- (0.81)
3. Heavy metals	
(a) Hg^{2+}	IO_4^- (0.05)-NO_2^- (0.92)
	IO_4^- (0.08)-Br^- (0.83)
(b) Pb^{2+}	IO_4^- (0.03)-NO_2^- (0.90)
	IO_4^- (0.01)-Br^- (ND)
(c) Ag^+	IO_4^- (0.05)-NO_2^- (0.94)
	IO_4^- (0.05)-Br^- (ND)
(d) Bi^{3+}	IO_4^- (0.04)-NO_2^- (0.94)
	IO_4^- (0.06)-Br^- (ND)

[a]Standard R_F values of individual ions are: IO_4^- (0.00), NO_2^- (0.92), and Br^- (0.90).
[b]ND = not detected.

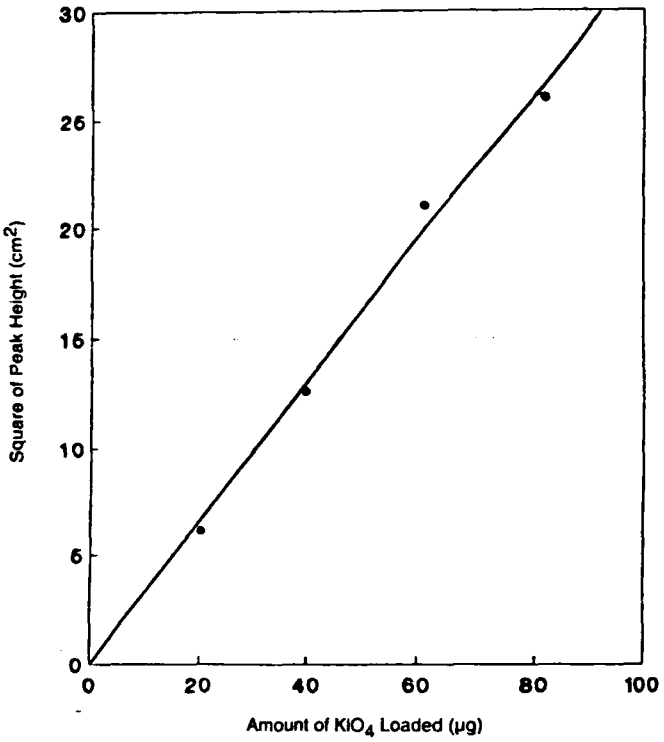

FIG. 1. Standard calibration curve for semiquantitative determination of IO_4^- by peak height measurement method.

tions of KIO_4 (0.02–0.1%) were spotted on kieselguhr thin layers. The chromatograms were developed and detected. The spots obtained were copied directly onto tracing paper from the chromatoplates. A linear relationship was obtained when the square of peak heights of the spots was plotted against microgram quantities of KIO_4 (Fig. 1).

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REFERENCES

1. Shinoda, K., and S. Frieberg, *Adv. Colloid Interface Sci. 4*:241 (1975).
2. Prince, L.M., *Microemulsions: Theory and Practice*, Academic Press, New York, 1977.
3. Robinson, B.H., *Chem. Br. 26*:342 (1990).
4. Berthod, A., and M.D. Carvalho, *Anal. Chem. 64*:2267 (1992).
5. Armstrong, D.W., and S.J. Henry, *J. Liq. Chromatogr. 3*:657 (1980).
6. Clive Love, L.J., J.G. Habarta, and J.G. Dorsey, *Anal. Chem. 56*:1132A (1984).
7. Hernandez-Torres, M.A., J.S. Landy, and J.G. Dorsey, *Ibid. 58*:744 (1986).
8. Armstrong, D.W., and W.L. Hinze, *Use of Ordered Media in Chemical Separations*, ACS Symposium series 342, American Chemical Society, Washington, D.C., 1987.
9. Berthod, A., O. Nicolas, and M. Porthault, *Anal. Chem. 62*:1402 (1990).
10. Kumar, S., and H.N. Singh, *Colloids Surfaces 44*:17 (1990).
11. Kumar, S., and H.N. Singh, *J. Surf. Sci. Technol. 5*:60 (1989).
12. Mohammad, A., and S. Tiwari, *Microchem. J. 44*:39 (1991).
13. Mohammad, A., and S. Tiwari, *Ibid. 47*:379 (1993).
14. Mohammad, A., S. Tiwari, and J.P.S. Chahar, *Chromatogr. Sci. 33*:143 (1995).
15. Souteyrand, C., M. Thibert, M. Caude, and R.J. Rosset, *Chromatogr. 316*:373 (1984).
16. Fendler, J.H., *Chem. Rev. 87*:877 (1987).
17. Fendler, J.H., *Acc. Chem. Res. 9*:153 (1976).

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Thin-layer chromatographic separation, colorimetric determination and recovery of thiocyanate from photogenic waste, river and sea waters

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JOURNAL OF CHROMATOGRAPHY A

Thin-layer chromatographic separation, colorimetric determination and recovery of thiocyanate from photogenic waste, river and sea waters

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Abstract

TLC was used to study the retention behaviour of some inorganic anions (SCN^-, NO_2^-, MnO_4^-, CrO_4^{2-}, $Cr_2O_7^{2-}$, MoO_4^{2-}, $Mo_7O_{24}^{6-}$, $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$) and metal ions (Fe^{3+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, Hg^{2+}, UO_2^{2+} VO^{2+}, Ag^+, Mn^{2+} and Bi^{3+}) on cellulose, Kieselguhr and their mixtures. Mixed solvent systems containing 0.1 *M* NH_4OH and CH_3COCH_3 were used as eluents. SCN^- separated from other ions was determined by spectrophotometry at 460 nm using acidic $FeCl_3$ solution as chromogenic reagent. Beer's law was followed up to 11.84 ppm of SCN^-. The proposed TLC-colorimetric method was applied to fortified samples of photogenic waste, river and seawaters.

Keywords: Water analysis; Environmental analysis; Thiocyanate; Inorganic anions; Inorganic cations

1. Introduction

Identification, separation and quantification of SCN^- is important due to its application in photography, catalysis, agri-chemicals, rust-inhibition and dyeing–printing of textiles [1]. At high acidities, thiocyanate produces cyanides in the presence of oxidants and thus causes lethal damage to aquatic life when SCN containing waste is discharged into rivers. Numerous methods [2–8] have been developed for analysing SCN^- and CN^- but most of them were not applied to polluted water and effluents. A spectrophotometric method based on the colour reaction of Fe^{3+} with SCN^- has been used for the quantification of SCN^- but it suffers from cationic, anionic and phenolic interferences. An extensive literature survey on the quantitative determination of SCN^- (1981–1992) with respect to the techniques used, shows that out of twenty research papers published, only one dealt with spectrophotometry.

Thin-layer chromatography (TLC) has been a widely used technique for the separation of SCN^- [9–12]. The literature survey [13] shows that out of forty research papers published so far on TLC of SCN^-, none examined the separation of SCN^- from complexing cations (Ag^+, Fe^{3+}, Co^{2+}, Cu^{2+} etc.). This paper reports a reliable TLC-colorimetric method for the determination of SCN^- in the presence of cationic and anionic impurities. The proposed meth-

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od is applicable to the determination of SCN^- in photogenic waste, river water and seawater.

2. Experimental

2.1. Apparatus

TLC applicator (Toshniwal, India); 20×3.5 cm glass plates, 24×6 cm jars, spectrophotometer (Elico, India, Ultra Spec. Model CL-54) and pH meter (Elico, India, Model LI-10T) were used.

2.2. Test solutions

Aqueous solutions (1.0%, w/v) of following were used.

1. Potassium salts of $Fe(CN)_6^{3-}$, $Fe(CN)_6^{4-}$, MnO_4^-, CrO_4^{2-} and $Cr_2O_7^{2-}$
2. Sodium salts of NO_2^- and MoO_4^{2-}
3. Ammonium salts of SCN^- and $Mo_7O_{24}^{6-}$
4. Nitrates of Ag^+, Bi^{3+}, UO_2^{2+} and Zn^{2+}
5. Chlorides of Fe^{3+} and Hg^{2+}
6. Sulphates of Cu^{2+}, VO^{2+}, Co^{2+} and Mn^{2+}

2.3. Chemicals

Ammonium thiocyanate (Merck, India), ferric chloride and ammonia solution (Qualigens), acetone, cellulose microcrystalline and Kieselguhr G (CDH, India) were used. All other chemicals were of analytical reagent grade.

2.4. Detection reagents

To locate the spots of analyte ions, the reagents used were:

1. 0.3% Diphenylamine in 4 *M* H_2SO_4 for NO_2^-, MnO_4^-, CrO_4^{2-} and $Cr_2O_7^{2-}$
2. 8.0% $FeCl_3$ in 2 *M* HCl for SCN^-, $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$
3. 0.5% Pyrogallol in methanol for MoO_4^{2-} and $Mo_7O_{24}^{6-}$
4. 1.0% $K_4Fe(CN)_6$ in water for Fe^{3+}, Cu^{2+}, VO^{2+} and UO_2^{2+}
5. 1.0% Dimethylglyoxime in ethanol for Co^{2+}
6. 1.0% Dithizone in benzene for Zn^{2+} and Cd^{2+}
7. Saturated H_2S in water for Ag^+, Hg^{2+} and Bi^{3+}

2.5. Stationary phases

Plain cellulose microcrystalline (S_1), Kieselguhr G (S_5) and their mixtures in 4:1 (S_2), 3:2 (S_3) and 1:1 (S_4) ratio were used as adsorbent materials.

2.6. Mobile phases

Mixtures of 0.1 *M* NH_4OH and CH_3COCH_3 in 1:9 (M_1), 3:7 (M_2), 5:5 (M_3), 7:3 (M_4) and 9:1 (M_5) ratios were used as solvent systems.

2.7. Preparation of chromatoplates

Cellulose, Kieselguhr G or their mixture was slurried with double distilled water in 1:3 and the resultant slurry (without additional binder) was applied onto glass plates with the aid of an applicator to give a layer of 0.25-mm thickness. Plates were dried at room temperature, activated at 100±2°C and then stored in a desiccating chamber until use.

2.8. Preparation of fortified samples

Four samples of SCN^- solutions (5.09–12.92 m*M*) were prepared in seawater (collected from Anjuna Beach, Goa). Similarly, spiked river water (Ganga, Rajghat) and photogenic waste (Aligarh) were prepared to examine the recovery of SCN^-.

2.9. Procedure

The entire methodology was carried out as follows

2.9.1. Chromatography of inorganic ions

To study the retention behaviour of some inorganic ions, 0.01 ml of the test solution was spotted onto the TLC plate, air dried and then developed with M_1–M_5 up to 10 cm. The plate was air dried, analyte ions were detected as colored spots and then identified on the basis of their R_F values. For mutual separations, an aliquot (0.02 ml) of binary or 0.03 ml of ternary mixture of analytes was loaded onto the plate and the chromatography was performed. In order to examine the effect of sample pH on the separation of SCN^-, various mixtures of test solu-

tions were brought to the required pH values (3.0–12.25) by adding either glacial acetic acid or NaOH solution. The effects of amines (2-nitroaniline, β-naphthylamine and diphenylamine) and phenols (phenol, resorcinol and pyrogallol) were examined on the detection and separation of SCN^- by mixing 1% alcoholic solution of additive with binary/ternary mixture containing SCN^-.

2.9.2. Colorimetry of thiocyanate

For quantitative determination of SCN^- by TLC-colorimetry, 0.1 ml of standard ammonium thiocyanate (2.55–25.48 m*M* SCN^-) was spotted onto the plate (S_2) along with foreign ions and developed with M_1. A pilot plate was simultaneously run. SCN^- containing portion of adsorbent from the working plate was scraped off and SCN^- was extracted with 10 ml of distilled water followed by washing of the adsorbent with 2 ml of water. Thus, the total volume of solution was kept to 12 ml in each case. To the filtrate, 0.5 ml of chromogenic reagent solution (8.0% ferric chloride in 2 *M* HCl) was added, absorbance was measured at 460 nm and the absorbance was plotted against the concentration (ppm) of SCN^- solution to sketch the standard calibration curve which was used to determine the concentration of SCN^- in a water sample with preliminary separation from accompanying inorganic ions.

2.9.3. Recovery from fortified samples

For the recovery of SCN^- from spiked waste (photogenic) and water (river and sea) samples, 0.1 ml of the solution was spotted onto TLC plate (chromatographic system; S_2, M_1) and the chromato-colorimetry was performed in the similar manner as that for standards. The loaded and recovered amounts of SCN^- were compared and the relative standard deviation (%), relative error and percentage recovery were calculated.

3. Results and discussion

3.1. Qualitative studies

Inorganic ions were chromatographed on plain and mixed adsorbents containing cellulose and/or Kieselguhr (S_1–S_5) using mixtures of 0.1 *M* NH_4OH and CH_3COCH_3 in various ratios as mobile phases (M_1–M_5). The results of this study are summarized in Tables 1 and 2. The effect of proportional composition of mobile phase on the hR_F values of inorganic anions is examined and the results are reported in Table 1 where only plain adsorbents (S_1 and S_5) were used. Cations were also chromatographed with these chromatographic systems, cations remain at the point of application. However, some metal ions yield occasional tailed spots. To achieve difference in migration of anions, S_1 and S_5 were mixed together to get a set of adsorbents (S_2–S_4) on which the anions were chromatographed using M_1 as mobile phase. The hR_F values of anions are enlisted in Table 2. Out of three mixed adsorbents, cellulose–Kieselguhr (4:1, S_2) is selected because it provides sufficient difference in hR_F values of SCN^- and others. It also provides compact spots because increasing Kieselguhr proportion results in the tailing of spots. Hence, the combination of S_2 and M_1 is the best chromatographic system which is utilized for the selective separation of SCN^- from all ions studied. These ions strongly interfere in the colorimetric

Table 1
Effect of mobile phase composition on the mobility of inorganic ions[a]

Anion	Stationary phase	hR_F values achieved with				
		M_1	M_2	M_3	M_4	M_5
SCN^-	S_1	49	91	91	93	95
	S_5	85	90	90	90	92
NO_2^-	S_1	21	76	82	91	93
	S_5	88	89	90	90	93
MnO_4^-	S_1	0	0	0	0	0
	S_5	0	0	0	0	0
$Fe(CN)_6^{3-}$	S_1	6	54	89	92	95
	S_5	83	90	90	92	93
$Fe(CN)_6^{4-}$	S_1	0	6	78	90	96
	S_5	8	17	80	91	93
CrO_4^{2-}	S_1	10	23	70	90	95
	S_5	41	91	91	93	94
$Cr_2O_7^{2-}$	S_1	8	20	78	88	92
	S_5	43	89	90	90	94
MoO_4^{2-}	S_1	9	24	83	90	93
	S_5	36	88	90	91	96
$Mo_7O_{24}^{6-}$	S_1	12	30	80	92	92
	S_5	38	90	91	95	95

[a] Metal ions remain at the point of application in all of the above mentioned chromatographic systems.

Table 2
Effect of stationary phase composition (cellulose/Kieselguhr proportion) on the mobility of inorganic ions[a] when developed with M_1

Anion studied	hR_F values achieved on				
	S_1	S_2	S_3	S_4	S_5
SCN^-	49	79	80	85	85
NO_2^-	21	21	32	86	88
MnO_4^-	0	0	0	0	0
$Fe(CN)_6^{3-}$	6	6	7	12	83
$Fe(CN)_6^{4-}$	0	0	0	0	0
CrO_4^{2-}	10	20	24	28	41[b]
$Cr_2O_7^{2-}$	8	19	19	25	43
MoO_4^{2-}	9	11	17	21	36[b]
$Mo_7O_{24}^{6-}$	12	15	15	23	38

[a] All metal ions studied remain at the point of application (hR_F = 0.0).
[b] Tailed spots ($hR_L - hR_T > 30$).

method of SCN^- quantification due to the formation of coloured complexes either with SCN^- or Fe^{3+} [14]. For Ag^+–SCN^- separation, the white precipitate of AgSCN formed on mixing of both ions was dissolved in NH_3 solution and the clear solution was spotted on the TLC plate. Fe^{3+}–SCN^- separation is of great importance, especially when SCN^- is to be determined colorimetrically by using $FeCl_3$ as chromogenic reagent.

For this separation, 1.0% aqueous NaF solution was added to red coloured complex to decolourize it. FeF_3, a more stable complex was formed and SCN^- became free and was detected on TLC plate. In the Co^{2+}–SCN^- separation, Co^{2+} could not be detected. In general, for metal ion–SCN^- separation, the amount of SCN^- was taken x times more than that of metal ion (x is the number of unit charges present on the metal ion). Effects of sample pH, phenols and amines on the detection of SCN^- have been examined and SCN^- was clearly detected in all cases. The R_F value of SCN^- remained unchanged over the pH range (3.0–12.25) of sample solution. No change in the mobility of SCN^- was noticed on being chromatographed in the presence of amines and phenols.

3.2. Quantitative studies

The proposed TLC system (S_2, M_1) was coupled to the spectrophotometry of SCN^- using Fe^{3+}–SCN^- colour reaction in an acidic (2 *M* HCl) medium. The complex absorbs incident radiation of 460 nm and follows the Beer's law up to 11.48 ppm. The optical density (A) plotted against the concentration (ppm) of SCN^- is an average of A_1 (of SCN^- after separation from NO_2^-), A_2 (from CrO_4^{2-}) and A_3 (from $Fe(CN)_6^{3-}$). The R.S.D. varies from 2.124–0.442%.

To test the applicability, the proposed TLC-colorimetric method was applied to the fortified

Table 3
Recovery of SCN^- from fortified environmental samples

Sample (pH)	Amount of SCN^- loaded (μg)	Amount of SCN^- recovered (μg)	Relative recovery (%)	Relative standard deviation (%)
Photogenic waste	29.60	30.19	102.00	9.38
(9.4)	44.40	45.48	102.44	5.60
	59.20	60.64	102.44	5.42
	74.00	76.46	103.32	10.70
River water	29.60	28.12	94.99	9.91
(8.2)	44.40	42.62	96.00	7.83
	59.20	57.22	96.66	7.09
	74.00	73.03	98.69	5.63
Sea water	29.60	27.38	92.50	9.91
(8.0)	44.40	41.64	93.78	7.57
	59.20	56.23	94.99	6.59
	74.00	71.53	96.66	5.45

samples of photogenic waste, river water and sea water. The results are summarized in Table 3. This table shows that the relative recoveries of SCN^- are almost the same.

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References

[1] C.J. Nicolas and A.J. Bernard, Anal. Chem., 55(11) (1983) 1667–82.
[2] W.J. Williams, in Handbook of Anion Determination, Butterworth, London, 1979, pp. 228–235.
[3] L. Zhihong, T. Shizong and C. Jieke, Sepu, 7 (1989) 44–6.
[4] A. Mohammad and S. Tiwari, Chromatographia, 30 (1990) 405–9.
[5] T. Shinzo, T. Toshihiko, O. Koreharu, T. Kiyomi and I. Toshio, Bunseki Kagaku, 30 (1981) 30–5.
[6] T. Akira, D. Kazuhiro and D. Toshio, Anal. Chim. Acta, 261 (1992) 281–6.
[7] C.S. Hwei, W.H. Lung, T. Minoru, S. Toshiyuki and F. Koichi, J. Chromatogr., 502 (1990) 257–264.
[8] I. Toshio, T. Shinzo, T. Toshihiko and T. Kawanishi, J. Chromatogr., 250 (1992) 55–61.
[9] J. Franc and E. Kosikova, J. Chromatogr., 187 (1980), 462–5.
[10] U.A.Th. Brinkman, G. De Vries and R. Kuroda, J. Chromatogr., 85 (1973) 187.
[11] K.G. Varshney and A. Mohammad, in J. Sherma and B. Fried (Editors), Handbook of Thin-Layer Chromatography 55, Marcel Dekker, New York, 1990, pp. 463–539.
[12] J. Sherma, Anal. Chem., 64 (1992) 134R–47R.
[13] A. Mohammad and S. Tiwari, Sep. Sci. Technol., 30 (19) (1995) 3577–3614.
[14] G. Svehla, Vogel's Quantitative Inorganic Analysis, Orient Longman, New Delhi, 6th ed., 1987, pp. 167–9.